VERIFICATION OF TRANSLATION

I, Mikiko Oyanagi

of Shimizu Patent Office, 6th Fl., Kantetsu Tsukuba-Science-City Bldg. 1-1-1, Oroshi-machi, Tsuchiura, Ibaraki, JAPAN 300-0847

declare as follows:

1. That I am well acquainted with both the English and Japanese languages, and

2. That the attached document is a true and correct translation of a certified copy of the following application, which was made by me to the best of my knowledge and belief:

(a) Japanese Patent Application No. 2002-339418
Entitled: "METHOD OF SCREENING FOR COMPOUNDS THAT INHIBIT THE ENZYMATIC ACTIVITY OF GWT1 GENE PRODUCT"
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M. Oyanagi
(Signature of Translator)
Mikiko Oyanagi

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【Addressee】 Esq. Commissioner of the Patent Office

【Inventor】

【Address】 20-6, Matsushiro 2-chome, Tsukuba-shi, IBARAKI

【Name】 Katsura Hata

【Inventor】

【Address】 4-2, Ninomiya 4-chome, Tsukuba-shi, IBARAKI

【Name】 Kappei Tsukahara

【Inventor】

【Address】 c/o Tsukuba Center NATIONAL INSTITUTE OF ADVANCED INDUSTRIAL SCIENCE AND TECHNOLOGY, 1-1-1, Higashi, Tsukuba-shi, IBARAKI

【Name】 Yoshifumi Jigami

【Inventor】

【Address】 c/o Tsukuba Center NATIONAL INSTITUTE OF ADVANCED INDUSTRIAL SCIENCE AND TECHNOLOGY, 1-1-1, Higashi, Tsukuba-shi, IBARAKI

【Name】 Kenichi Nakayama

【Inventor】

【Address】 c/o Tsukuba Center NATIONAL INSTITUTE OF ADVANCED INDUSTRIAL SCIENCE AND TECHNOLOGY, 1-1-1, Higashi, Tsukuba-shi, IBARAKI

【Name】 Mariko Umemura

【Inventor】

【Address】 c/o Tsukuba Center NATIONAL INSTITUTE OF ADVANCED INDUSTRIAL SCIENCE AND TECHNOLOGY, 1-1-1, Higashi, Tsukuba-shi, IBARAKI

【Name】 Michiyo Okamoto

【Applicant】

【Identification Number】 000000217

【Name or Appellation】 EISAI CO., LTD.

【Applicant】

【Identification Number】 301021533

【Name or Appellation】 National Institute of Advanced Industrial Science and Technology

【Attorney】

【Identification Number】 100102978

【Patent Attorney】

【Name or Appellation】 Hatsushi Shimizu

【Nominated Attorney】

【Identification Number】 100108774

【Patent Attorney】

【Name or Appellation】 Kazunori Hashimoto

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【List of Attached Documents】

【Name of Document】 Description 1

【Name of Document】 Drawings 1

【Name of Document】 Abstract 1

【Proof】 Necessary

[Document Name] Specification

[Title of the Invention] METHOD OF SCREENING FOR COMPOUNDS THAT INHIBIT THE ENZYMATIC ACTIVITY OF GWT1 GENE PRODUCT

[Claims]

[Claim 1] A method of screening for a compound having an antifungal activity, wherein the method comprises the steps of:

(1) contacting a test sample with an overexpressed protein encoded by the GWT1 gene;

(2) detecting GlcN-(acyl)PI; and

(3) selecting the test sample that decreases GlcN-(acyl)PI.

[Claim 2] The method of claim 1, wherein the GWT1 gene is any one of the following:

(a) a DNA encoding a protein comprising the amino acid sequence of SEQ ID NO: 2, 4, 6, 8, 10, or 14;

(b) a DNA comprising the nucleotide sequence of SEQ ID NO: 1, 3, 5, 7, 9, 11, 12, or 13;

(c) a DNA hybridizing to the DNA comprising the nucleotide sequence of SEQ ID NO: 1, 3, 5, 7, 9, 11, 12, or 13 under stringent conditions; and

(d) a DNA encoding a protein comprising the amino acid sequence of SEQ ID NO: 2, 4, 6, 8, 10, or 14, wherein one or more amino acids have been added, deleted, substituted, and/or inserted.

[Claim 3] The method of claim 1 or 2, wherein the step of detecting the acylated GPI is thin-layer chromatography.

[Claim 4] The method of any one of claims 1 to 3, wherein the method further comprises a step 4, of determining whether the selected test sample inhibits the process of transporting a GPI-anchored protein to a fungal cell wall, whether the test sample inhibits the expression of a GPI-anchored protein on a fungal cell surface, or whether the test sample inhibits the proliferation of a fungi.

[Detailed Description of the Invention]

[0001] [Technical Field of Industrial Application]

The present invention relates to methods of screening for antifungal agents having the

activity of inhibiting GPI synthase, which is involved in the synthesis of fungal cell walls.

[0002] [Prior Art]

The present inventors noticed that adhesion to host cells is important for fungi to exert their pathogenicity, and that adhesion factors involved in fungal cell adhesion are transported to the surface layers of cell walls after glycosylphosphatidylinositol (GPI) anchors on the cell membrane (Non-Patent Document 1). Accordingly, the present inventors considered that novel antifungal agents that inhibit the synthesis of fungal cell walls and also inhibit the adhesion of fungal cells to host cells could be generated by inhibiting the process of transporting proteins anchored with GPI (GPI-anchored proteins) to cell walls. Thus, the present inventors started study.

[0003] The prior art reference related to the invention of the present application is shown below:

[Non-Patent Document 1] Hamada K *et al.*, Mol. Gen. Genet., 258: 53-59, 1998

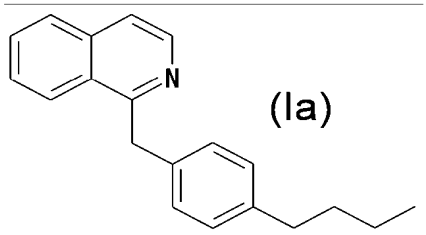
[0004] [Problems to be Solved by the Invention]

An objective of the present invention is to develop antifungal agents for preventing pathogenic fungi from exerting pathogenicity, by inhibiting the synthesis of fungal cell walls, as well as by inhibiting fungal cell adhesion to host cells, by inhibition of the transport of GPI-anchored proteins to fungal cell walls.

[0005] [Means to Solve the Problems]

In WO 02/04626, the present inventors found the following proteins involved in the process of transporting GPI-anchored proteins to cell walls: the proteins of *Saccharomyces cerevisiae* encoded by DNAs comprising the nucleotide sequence of SEQ ID NO: 1; the proteins of *Candida albicans* encoded by DNAs comprising the nucleotide sequences of SEQ ID NOs: 3 and 5; the proteins of *Schizosaccharomyces pombe* encoded by DNAs comprising the nucleotide sequence of SEQ ID NO: 7; the proteins of *Aspergillus fumigatus* encoded by DNAs comprising the nucleotide sequences of SEQ ID NOs: 9 and 11; and the proteins of *Cryptococcus neoformans* encoded by DNAs comprising the nucleotide sequences of SEQ ID NOs: 12 and 13. These nucleotide sequences were called GWT1 genes. In addition, the inventors found that GWT1 gene-deficient fungi can not synthesize cell walls. Furthermore, the inventors found that the compound represented by formula (Ia) binds to the above-described proteins to inhibit the transport of GPI-anchored proteins to cell walls, thus inhibiting the synthesis of fungal cell walls.

[0006] [Compound 1]



(Ia)

[0007] The inventors then found that the GWT1 gene product (hereinafter referred to as "GWT1 protein") has the activity of synthesizing GlcN-(acyl)PI by transferring an acyl group to GlcN-PI in the GPI biosynthesis pathway (Fig. 1; Kinoshita and Inoue, Curr Opin Chem Biol 2000 Dec;4(6): 632-8; Ferguson *et al.*, Biochim Biophys Acta 1999 Oct 8; 1455 (2-3): 327-40). The inventors conceived that compounds inhibiting the synthesis of fungal cell walls could be found by screening for compounds that inhibit this activity, and thus completed the present invention.

[0008] Specifically, the present invention provides 1 to 7 as described below.

1. A method of screening for a compound having an antifungal activity, wherein the method comprises the steps of:

(1) contacting a test sample with an overexpressed protein encoded by the GWT1 gene;

(2) detecting GlcN-(acyl)PI; and

(3) selecting the test sample that decreases GlcN-(acyl)PI.

[0009] The "GWT1" gene refers to a gene involved in the synthesis of fungal cell walls, which was disclosed in WO 02/04626. The term "overexpressed" does not refer to expression of native genes, but to the expression of exogenously introduced genes.

[0010] "GlcN-(acyl)PI" refers to glucosaminyl-acylphosphatidylinositol in which an acyl group is linked with the inositol of glucosaminyl-phosphatidylinositol (GlcN-PI) in the GPI biosynthesis pathway (Fig. 1; Kinoshita and Inoue, Curr Opin Chem Biol 2000 Dec; 4(6):632-8; Ferguson *et al.*, Biochim Biophys Acta 1999 Oct 8; 1455(2-3):327-40).

[0011] 2. The method of claim 1, wherein the GWT1 gene is any one of the following:

(a) a DNA encoding a protein comprising the amino acid sequence of SEQ ID NO: 2, 4, 6, 8, 10, or 14;

(b) a DNA comprising the nucleotide sequence of SEQ ID NO: 1, 3, 5, 7, 9, 11, 12, or 13;

(c) a DNA hybridizing to the DNA comprising the nucleotide sequence of SEQ ID NO: 1, 3, 5, 7, 9, 11, 12, or 13 under stringent conditions; and

(d) a DNA encoding a protein comprising the amino acid sequence of SEQ ID NO: 2, 4, 6, 8, 10, or 14, wherein one or more amino acids have been added, deleted, substituted, and/or inserted.

[0012] The term "stringent conditions" means, for example, hybridization in 4x SSC at 65°C followed by washing with 0.1x SSC at 65°C for one hour. Alternatively, stringent conditions refer to hybridization in 4x SSC with 50% formamide at 42°C. Other acceptable conditions may be hybridization in PerfectHyb™ solution (TOYOBO) at 65°C for 2.5 hours, followed by washing with (1) 2x SSC, 0.05% SDS at 25°C for five minutes; (2) 2x SSC, 0.05% SDS at 25°C for 15 minutes; and (3) 0.1x SSC, 0.1% SDS at 50°C for 20 minutes.

[0013] The "protein comprising an amino acid sequence in which one or more amino acids have been added, deleted, substituted, and/or inserted" can be prepared by methods known to those skilled in the art, for example, by site-directed mutagenesis (Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY). Such mutations can also occur naturally. There is no limitation on the number of amino acids to be mutated, as long as the binding activity with the labeled compound is maintained. The number of amino acids to be mutated is typically 30 or less, preferably ten or less, and more preferably three or less. There is no limitation on the position of the mutated amino acids, as long as the protein retains the activity described above.

[0014] The proteins and protein mutants prepared using the above-described hybridization techniques normally have high homology (for example, 60% or higher, 70% or higher, 80% or higher, 90% or higher, or 95% or higher homology) to proteins consisting of the amino acid sequence of SEQ ID NO: 2, 4, 6, 8, 10, or 14 at the amino acid level. The amino acid sequence homology can be determined using a BLASTx program (at the amino acid level; Altschul *et al.*, J. Mol. Biol. 215:403-410, 1990). This program is based on the BLAST algorithm of Karlin and Altschul (Proc. Natl. Acad. Sci. USA 87:2264-2268, 1990; Proc. Natl. Acad. Sci. USA 90:5873-5877, 1993). When the amino acid sequences are analyzed using BLASTX, parameters of, for example, score= 50 and wordlength= 3 are used. Alternatively, when using the Gapped BLAST program, the amino acid sequences can be analyzed by the method described by Altschul *et al.* (Nucleic. Acids. Res. 25:3389-3402, 1997). When the BLAST and Gapped BLAST programs are used, the default parameter values for each program are used. Specific procedures for these analyses are known in the art (http://www.ncbi.nlm.nih.gov).

[0015] 3. The method of claim 1 or 2, wherein the step of detecting the acylated GPI is thin-layer chromatography.

4. The method of any one of claims 1 to 3, wherein the method further comprises a step 4, of determining whether the selected test sample inhibits the process of transporting a GPI-anchored protein to a fungal cell wall, whether the test sample inhibits the expression of a GPI-anchored protein on a fungal cell surface, or whether the test sample inhibits the proliferation of a fungi.

[[0016] [Mode for Carrying Out the Invention]

Methods for preparing GWT1 protein [1], and methods for determining transacylation activity [2] of the present invention are disclosed below.

1. Methods for preparing GWT1 protein

GWT1 protein is prepared from a fungal membrane fraction, preferably that of *S. cerevisiae*, *C. albicans*, *S. pombe*, *A. fumigatus*, or *C. neoformans*, and more preferably *S. cerevisiae*. The transacylation activity may be determined by using the prepared membrane fraction directly or after purification. The transacylation activity can be readily measured by introducing a DNA of the nucleotide sequence of SEQ ID NO: 1, 3, 5, 7, 9, 11, 12, or 13 into fungal cells to overexpress the GWT1 protein. This procedure can be specifically described using *S. cerevisiae*, as follows:

[0017] (1) Introduction of the GWT1 gene

The GWT1 gene can be prepared by carrying out PCR using fungal DNAs as templates, and primers designed based on a nucleotide sequence of SEQ ID NO: 1, 3, 5, 7, 9, 11, 12, or 13.

The GWT1 expression plasmid is prepared by inserting an appropriate promotor and terminator, such as a GAPDH promoter and a GAPDH terminator derived from pKT10 (Tanaka *et al.*, Mol. Cell Biol., 10: 4303-4313, 1990), into the multi-cloning site of an expression vector that functions in *S. cerevisiae,* such as YEp352, and inserting the GWT1 gene into the expression vector. *S. cerevisiae* cells of, for example, G2-10 strain, are incubated while shaking in an appropriate medium such as yeast extract-polypeptone-dextrose (YPD) medium at an appropriate temperature, for example, at 30°C. The fungal cells are harvested at the late logarithmic growth phase. After washing, GWT1 expression plasmids are introduced into *S. cerevisiae* cells, for example, by the lithium acetate method. The lithium acetate method is described in the Users Manual attached to YEAST MAKER™ Yeast Transformation System (Clontech). GWT1-overexpressing strain and empty vector-introduced strain can be obtained by culturing the cells in SD(ura-) medium at 30°C for two days.

[0018] Fungal strains to which the GWT1 gene is introduced are preferably deficient strains lacking their native GWT1 gene. *S. cerevisiae* GWT1 gene-deficient cells can be obtained by a method described below.

PCR amplification is carried out using a marker gene, preferably *S. pombe* his5 gene, as a template, and primers designed to obtain PCR products which comprise 30 bp, or more preferably 40 bp or more of the GWT1 gene sequence (for example, the sequence of SEQ ID NO: 1) to be deleted. The resulting PCR products are purified, and then introduced into fungal

cells. Deficient strains can be obtained by screening appropriate to the marker gene, for example, by culturing the cells in his- medium when the marker is his5.

[0019] Expression vectors and gene introduction methods for fungus other than *S. cerevisiae* are described in: Igarashi *et al.*, Nature 353: 80-83, 1991, for *S. pombe* expression vector pcL and such, and methods for introducing the vectors; Pla J *et al.*, Yeast, 12: 1677-1702, 1996, for *C. albicans* expression vector pRM10 and such, and methods for introducing these vectors; Punt PJ *et al.*, GENE, 56: 117-124, 1987, for *A. fumigatus* expression vector pAN7-1 and such, and methods for introducing these vectors; and Monden P *et al.*, FEMS Microbiol. Lett., 187: 41-45, 2000, for *C. neoformans* expression vector pPM8 and such, and methods for introducing these vectors.

Methods for preparing deficient strains of *C. albicans* are described in Fonzi WA *et al.*, Genetics 134: 717-728, 1993.

[0020] (2) Methods for preparing the membrane fraction

S. cerevisiae cells to which the GWT1 gene are introduced are cultured while shaking in an appropriate medium, such as SD(ura-) liquid medium, at an appropriate temperature, for example 24°C. The fungal cells are harvested in the middle logarithmic growth phase. After being washed with TM buffer (50 mM Tris-HCl (pH 7.5) and 2 mM $MgCl_2$), the fungal cells are suspended in an adequate amount (for example, 2 ml) of TM buffer + protease inhibitor (Complete™; Roche). An adequate amount (for example, 1.5 ml) of glass beads is added to the suspension. The samples are vortexed and placed on ice, and these procedures are repeated (for example, ten cycles of vortexing for 30 seconds and placing on ice for 30 seconds) to disrupt fungal cells.

The samples are centrifuged, for example, at 1000 g for five minutes, to precipitate glass beads and fungal cells which are not disrupted. The resulting supernatant is transferred to another tube, and then centrifuged, to precipitate the membrane fraction comprising organelles (total membrane fraction), for example at 13 000 g for 20 minutes. If required, the precipitate is further suspended in 1 ml of an appropriate assay buffer, and centrifuged, for example, at 1000 g for one minute to remove those components which are not suspended. The supernatant is then centrifuged, for example, at 13 000 g for 20 minutes, and the resulting precipitate is resuspended in an appropriate assay buffer to obtain a membrane fraction.

[0021] Membrane fractions from fungal cells other than *S. cerevisiae* can be prepared by the methods as described in: Yoko-o *et al.*, Eur. J. Biochem. 257: 630-637, 1998, for *S. pombe*; Sentandreu M *et al.*, J. Bacteriol., 180: 282-289, 1998, for *C. albicans*; Mouyna I *et al.*, J. Biol. Chem., 275: 14882-14889, 2000, for *A. fumigatus*; and Thompson JR *et al.*, J. Bacteriol., 181: 444-453, 1999, for *C. neoformans*.

[0022] Alternatively, GWT1 protein can be prepared by expression in cells other than fungal cells, such as mammalian cells, insect cells, and *E. coli* cells.

When mammalian cells are used, a membrane fraction can be prepared by inserting GWT1 into, for example, an overexpression vector comprising CMV promotor; introducing the vector into mammalian cells; and then carrying out the method described in Petaja-Repo *et al.*, J. Biol. Chem., 276: 4416-23, 2001.

[0023] When insect cells are used, a membrane fraction can be prepared by preparing GWT1-expressing insect cells (such as Sf9 cells) using a baculovirus expression kit, for example, BAC-TO-BAC Baculovirus Expression system (GIBCO BRL); and then using the cells to carry out the method described in Okamoto *et al.*, J. Biol. Chem., 276: 742-751, 2001.

When *E. coli* is used, GWT1 protein can be prepared by inserting GWT1 into an *E. coli* expression vector, for example, pGEX (Pharmacia); and then introducing the vector into *E. coli* cells such as BL21.

[0024] 2. Methods for determining transacylation activity

The transacylation reaction to GPI can be detected by the method described in Costello and Orlean, J. Biol. Chem. (1992) 267: 8599-8603, or the method described in Franzot and Doering, Biochem. J. (1999) 340: 25-32. Examples of specific methods are illustrated below, however, the experimental conditions below are preferably optimized according to the GWT1 gene products to be used, as follows:

The GWT1 gene product prepared in Section 1, above, preferably a membrane fraction comprising a GWT1 gene product, is added along with test compounds to a buffer comprising: appropriate metal ions (Mg, Mn); ATP; and Coenzyme A; and preferably inhibitors that prevent the consumption of UDP-GlcNAc in other reactions, such as nikkomycin Z as an inhibitor of chitin synthesis, and tunicamycin as an inhibitor of the synthesis of asparagine-linked sugar chain. The mixture is incubated at an appropriate temperature for an appropriate period (for example, at 24°C for 15 minutes).

[0025] Then, a GlcN-(acyl)PI precursor (for example, UDP-GlcNAc or Acyl-Coenzyme A, and preferably UDP-[^{14}C]GlcNAc) labeled with an appropriate label, preferably with an isotope, is added to the mixture. The resulting mixture is further incubated for an appropriate period (for example, for one hour at 24°C). A 1:2 mixture of chloroform: methanol is added to the mixture, and stirred to stop the reaction. Lipids are then extracted from the mixture. The extracted reaction products are dissolved in an appropriate solvent, preferably in butanol, and then subjected to HPLC, thin-layer chromatography (TLC), or such, and preferably TLC, to isolate GlcN-(acyl)PI generated in the reaction. A developing solvent for TLC can be selected appropriately, and may be, for example, $CHCl_3/CH_3OH/H_2O$ (65:25:4), $CHCl_3/CH_3OH$/1 M

NH_4OH (10:10:3), or $CHCl_3$/pyridine/HCOOH (35:30:7), and preferably HCl_3/CH_3OH/1 M NH_4OH (10:10:3). The isolated GlcN-(acyl)PI is quantified by a method that accords with the label used. When labeled with an isotope, the isolated GlcN-(acyl)PI is quantified based on its radioactivity.

When a reduced amount of GlcN-(acyl)PI is produced in the presence of a test compound, the test compound is determined to comprise the activity of inhibiting transacylation by GWT1 proteins.

[0026] A test sample found to comprise the activity of inhibiting transacylation as described above, is preferably further tested to determine whether it inhibits the process of transporting GPI-anchored proteins to fungal cell walls, whether it inhibits the expression of GPI-anchored proteins on fungal cell surfaces, or whether it inhibits fungal growth. If the test results show that the test sample inhibits the process of transporting GPI-anchored proteins to fungal cell walls, inhibits the expression of GPI-anchored proteins on fungal cell surfaces, or inhibits fungal growth, then the sample is a promising candidate for an antifungal agent.

[0027] Methods that (1) use reporter enzymes; (2) use antibodies that react to glycoproteins on the surface layers of fungal cell walls; (3) test fungal cells for adhesiveness to animal cells; or (4) observe fungal cells under a light microscope or electron microscope can be used to test whether a test sample inhibits the process of transporting GPI-anchored proteins to fungal cell walls or inhibits the expression of GPI-anchored proteins on fungal cell surfaces.

[0028] Methods (1) to (4) are enclosed in WO 02/04626, and specifically illustrated in the Examples. By using the methods of (1) to (4), preferably in combination, a test sample can be determined to inhibit the process of transporting GPI-anchored proteins to fungal cell walls or to inhibit the expression of GPI-anchored proteins on fungal cell surfaces. Further, a test sample can be determined to effect the process of transporting GPI-anchored proteins to cell walls, when the inhibition by the test sample is impaired or disappears when a protein encoded by a DNA of the present invention is overexpressed in fungal cells.

[0029] Conventional methods for measuring antifungal activity can also be used to determine whether a test sample inhibits fungal growth (National Committee for Clinical Laboratory Standards. 1992. Reference method for broth dilution antifungal susceptibility testing for yeasts. Proposed standard M27-P. National Committee for Clinical Laboratory Standards, Villanova, Pa.).

[0030] [Examples]

Herein below, the present invention will be specifically described using Examples, but it is not to be construed as being limited thereto.

[Example 1] Preparation of membrane fraction expressing GWT1 protein

(1) Preparation of GWT1 expression plasmid

The vector for expressing in *S. cerevisiae*, YEp352GAPII vector, was prepared by inserting a GAPDH promoter and a GAPDH terminator, both derived from pKT10 (Tanaka *et al.*, Mol. Cell Biol., 10: 4303-4313, 1990), into the multi-cloning site of YEp352; and replacing the multi-cloning site with that of pUC18. Furthermore, to facilitate the insertion of the GWT1 gene, YEp352GAPIIClaIΔSal vector was prepared by substituting the ClaI site for the SalI site in the multi-cloning site.

The *S. cerevisiae* GWT1 gene comprising the nucleotide sequence of SEQ ID NO: 1 was amplified using the primers of SEQ ID NOs: 15 and 16. The resulting PCR product was inserted into the multi-cloning site of YEp352GAPIIClaIΔSal vector to prepare the GWT1 overexpression plasmid.

[0031] (2) Preparation of *S. cerevisiae* GWT1 gene-deficient strain Δgwt1

A his5 cassette comprising GWT1 sequences at both ends was amplified by PCR using the *S. pombe* his5 gene (Longtine MS *et al.*, Yeast, 14: 953-961, 1998) as a template and the sequences of SEQ ID NOs: 17 and 18 as primers.

S. cerevisiae cells were cultured and harvested, and then subjected to transformation with the PCR products described above. Then, the cells were cultured in SD(His-) medium at 30°C for five to seven days to obtain GWT1 gene-deficient strain Δgwt1.

[0032] (3) Preparation of GWT1-expressing cells

Cells of the Δgwt1 strain were cultured while shaking in yeast extract-polypeptone-dextrose (YPD) medium at 30°C. The cells were harvested in the late logarithmic growth phase and then washed. The expression plasmid for GWT1 was introduced to the Δgwt1 strain cells by the lithium acetate method (YEAST MAKER™ Yeast Transformation System (Clontech)). Δgwt1 strain overexpressing the GWT1 gene was obtained by culturing the cells in SD(ura-) medium at 30°C for two days.

[0033] (4) Preparation of membrane fraction

Wild-type *S. cerevisiae* strain, the GWT1 gene-deficient strain Δgwt1, and the strain Δgwt1 into which the GWT1 overexpression plasmid was introduced were each cultured in 100 ml of YPD medium shaken at 24°C, and then harvested in the middle logarithmic growth phase (OD_{600}= 1 ~ 3). The fungal cells were washed with TM buffer (50 mM Tris-HCl (pH 7.5) and 2 mM $MgCl_2$), and then suspended in 2 ml of TM buffer + protease inhibitor (1 tablet of Complete™ (Roche) / 25 ml). 1.5 ml of glass beads was added to the suspension. The

mixture was vortexed for 30 seconds, and then placed on ice for 30 seconds. These procedures were repeated ten times to disrupt the fungal cells. The cell homogenate was transferred into a new tube, and centrifuged at 1000 g at 4°C for five minutes to precipitate the glass beads and undisrupted fungal cells. The supernatant was transferred to another tube, and centrifuged at 13 000g at 4°C for 20 minutes to precipitate the membrane fraction comprising organelles (total membrane fraction). The resulting precipitate was used as the membrane fraction.

[0034] (5) Detection of acylated GPI

In the GPI biosynthesis reaction pathway, it is known that N-acetyl-glucosaminyl-phosphatidylinositol (GlcNAc-PI) is deacetylated to generate glucosaminyl-phosphatidylinositol (GlcN-PI), to which an acyl group is then added to generate glucosaminyl-acylphosphatidylinositol (GlcN-(acyl)PI) (Fig. 1). The present inventors thus tested whether the Gwt1 protein was involved in this transacylation reaction using the method described below.

[0035] The membrane fraction preparation (300 μg protein) was diluted with a buffer consisting of 50 mM Tris-HCl (pH7.5), 2 mM $MgCl_2$, 2 mM $MnCl_2$, 1 mM ATP, 1 mM Coenzyme A, 21 μg/ml tunicamycin, 10 μM nikkomycin Z, and 0.5 mM Dithiothreitol. The solution was adjusted to a total of 140 μl for use as a reaction solution. After incubating the solution at 24°C for 15 minutes, 15 μCi UDP-[^{14}C]GlcNAc was added to the tube and then incubated at 24°C for another one hour. 1 ml of chloroform:methanol (1:2) was added to the solution and stirred to stop the reaction. Then, lipid was extracted from the solution, dried, and desalted by butanol extraction. Acylated GPI (GlcN-(acyl)PI), non-acylated GPI (GlcN-PI), and GPI which was neither acylated nor deacylated (GlcNAc-PI) were separated by thin-layer chromatography (HCl_3/CH_3OH/1 M NH_4OH (10:10:3)). Each spot was detected by autoradiography.

[0036] As a result, as shown in Fig. 2, a spot for acylated GPI was not detected in the GWT1 gene-deficient strain (Δgwt1), while it was detected in the wild-type strain. The spot for acylated GPI was also detected in the GWT1 gene-introduced Δgwt1 strain, showing that this strain had recovered ability to acylate. These findings indicate that the Gwt1 protein is an enzyme that catalyzes transacylation to GPI.

[0037] The above-described results suggest that the intensity of the spot for acylated GlcN-(acyl)PI is reduced or disappears when a compound having the activity of inhibiting the activity of GWT1 gene products is present in a system for assaying GPI synthase activity. Accordingly, compounds inhibiting the enzymatic activity of a GWT1 gene product, as well as compounds inhibiting the synthesis of fungal cell walls, can be screened using the intensity of GlcN-(acyl)PI spots as an indicator.

[0038] [Effects of the Invention]

The present invention makes it possible to screen for compounds that inhibit the transport of GPI-anchored proteins to fungal cell walls by using a simple assay of transacylation activity.

[0039] [Sequence Listing]

<110> Eisai Co., Ltd.
National Institute of Advanced Industrial Science and Technology

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<130>

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<170> PatentIn Ver. 2.0

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tat gct agt gaa cca tac ctt cta aac acg cta ata ctg tta cct tgt   288
Tyr Ala Ser Glu Pro Tyr Leu Leu Asn Thr Leu Ile Leu Leu Pro Cys
                 85                  90                  95
ttg ctc gca ttc ata tat gga aaa ttt act agc tcg agt aaa cct tct   336
Leu Leu Ala Phe Ile Tyr Gly Lys Phe Thr Ser Ser Ser Lys Pro Ser
            100                 105                 110
aat cca ata tac aat aaa aaa aaa atg att aca cag cgg ttc caa cta   384
Asn Pro Ile Tyr Asn Lys Lys Lys Met Ile Thr Gln Arg Phe Gln Leu
        115                 120                 125
gaa aaa aag ccg tat att act gcg tat cgt ggt ggg atg ctt att ctg   432
Glu Lys Lys Pro Tyr Ile Thr Ala Tyr Arg Gly Gly Met Leu Ile Leu
    130                 135                 140
act gct att gcc atc ttg gct gta gat ttt cca att ttc cca agg agg   480
Thr Ala Ile Ala Ile Leu Ala Val Asp Phe Pro Ile Phe Pro Arg Arg
145                 150                 155                 160
ttt gcc aag gtg gaa act tgg ggg aca tcc ctg atg gat ctt ggt gta   528
Phe Ala Lys Val Glu Thr Trp Gly Thr Ser Leu Met Asp Leu Gly Val
                165                 170                 175
gga tca ttc gtt ttc agt aac ggt att gtt tct tct agg gca ctg ttg   576
Gly Ser Phe Val Phe Ser Asn Gly Ile Val Ser Ser Arg Ala Leu Leu
            180                 185                 190
aaa aac cta agc ttg aag agt aaa ccc agc ttc tta aaa aat gca ttt   624
Lys Asn Leu Ser Leu Lys Ser Lys Pro Ser Phe Leu Lys Asn Ala Phe
        195                 200                 205
aat gcc tta aaa tca gga gga act cta ttg ttc cta gga ttg ctg agg   672
Asn Ala Leu Lys Ser Gly Gly Thr Leu Leu Phe Leu Gly Leu Leu Arg
    210                 215                 220
ttg ttt ttt gta aaa aat ttg gaa tat caa gaa cat gtc aca gaa tat   720
Leu Phe Phe Val Lys Asn Leu Glu Tyr Gln Glu His Val Thr Glu Tyr
225                 230                 235                 240
ggg gtt cat tgg aat ttt ttt atc acc cta tca ttg ttg cca ctt gta   768
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Gly Val His Trp Asn Phe Phe Ile Thr Leu Ser Leu Leu Pro Leu Val
                245                 250                 255
ttg acc ttt att gat ccc gtc aca aga atg gtt cca cgc tgc tca att   816
Leu Thr Phe Ile Asp Pro Val Thr Arg Met Val Pro Arg Cys Ser Ile
            260                 265                 270
gca ata ttc att tca tgc att tat gaa tgg cta ctt tta aag gac gat   864
Ala Ile Phe Ile Ser Cys Ile Tyr Glu Trp Leu Leu Leu Lys Asp Asp
        275                 280                 285
cgc act tta aac ttt tta att ttg gct gat aga aat tgt ttc ttc agt   912
Arg Thr Leu Asn Phe Leu Ile Leu Ala Asp Arg Asn Cys Phe Phe Ser
    290                 295                 300
gct aat aga gaa ggc atc ttc tca ttt cta ggt tat tgc tcg att ttt   960
Ala Asn Arg Glu Gly Ile Phe Ser Phe Leu Gly Tyr Cys Ser Ile Phe
305                 310                 315                 320
ctt tgg ggc caa aac acg gga ttt tac ttg ttg gga aat aaa cca act   1008
Leu Trp Gly Gln Asn Thr Gly Phe Tyr Leu Leu Gly Asn Lys Pro Thr
                325                 330                 335
tta aac aat ctt tat aag cct tct acg caa gac gta gtt gca gca tca   1056
Leu Asn Asn Leu Tyr Lys Pro Ser Thr Gln Asp Val Val Ala Ala Ser
            340                 345                 350
aag aag tct tcg act tgg gac tat tgg act tca gta acc cca tta agt   1104
Lys Lys Ser Ser Thr Trp Asp Tyr Trp Thr Ser Val Thr Pro Leu Ser
        355                 360                 365
ggc ctc tgt ata tgg agt aca att ttt ctt gtt atc agc cag ttg gtt   1152
Gly Leu Cys Ile Trp Ser Thr Ile Phe Leu Val Ile Ser Gln Leu Val
    370                 375                 380
ttt caa tac cat cct tat agt gtt tca aga agg ttt gct aac tta cca   1200
Phe Gln Tyr His Pro Tyr Ser Val Ser Arg Arg Phe Ala Asn Leu Pro
385                 390                 395                 400
tat act ttg tgg gtc att act tat aat tta cta ttt ttg act ggg tac   1248
Tyr Thr Leu Trp Val Ile Thr Tyr Asn Leu Leu Phe Leu Thr Gly Tyr
                405                 410                 415
tgc ttg act gac aaa att ttc ggt aat tct tcg gaa tat tat aaa gtt   1296
Cys Leu Thr Asp Lys Ile Phe Gly Asn Ser Ser Glu Tyr Tyr Lys Val
            420                 425                 430
gcc gaa tgc ttg gaa tca atc aac tcc aat ggg ttg ttt tta ttt ttg   1344
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Ala Glu Cys Leu Glu Ser Ile Asn Ser Asn Gly Leu Phe Leu Phe Leu
435 440 445

ttg gca aat gtc tct act ggt tta gtc aat atg tct atg gtc acg ata 1392
Leu Ala Asn Val Ser Thr Gly Leu Val Asn Met Ser Met Val Thr Ile
450 455 460

gat tct tca ccc tta aaa tca ttc ctg gtt ttg ttg gca tac tgc tca 1440
Asp Ser Ser Pro Leu Lys Ser Phe Leu Val Leu Leu Ala Tyr Cys Ser
465 470 475 480

ttc ata gct gtc ata tcg gtt ttc ttg tat aga aaa aga ata ttc att 1488
Phe Ile Ala Val Ile Ser Val Phe Leu Tyr Arg Lys Arg Ile Phe Ile
485 490 495

aag cta taa 1497
Lys Leu

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<212> PRT
<213> Saccharomyces cerevisiae

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20 25 30
Ala Val Thr Ser Ile Ala Leu Val Thr Tyr Ile Ser Trp Asn Leu Leu
35 40 45
Lys Asn Ser Asn Leu Met Pro Pro Gly Ile Ser Ser Val Gln Tyr Ile
50 55 60
Ile Asp Phe Ala Leu Asn Trp Val Ala Leu Leu Leu Ser Ile Thr Ile
65 70 75 80
Tyr Ala Ser Glu Pro Tyr Leu Leu Asn Thr Leu Ile Leu Leu Pro Cys
85 90 95
Leu Leu Ala Phe Ile Tyr Gly Lys Phe Thr Ser Ser Ser Lys Pro Ser
100 105 110
Asn Pro Ile Tyr Asn Lys Lys Lys Met Ile Thr Gln Arg Phe Gln Leu

115 120 125

Glu Lys Lys Pro Tyr Ile Thr Ala Tyr Arg Gly Gly Met Leu Ile Leu
130 135 140

Thr Ala Ile Ala Ile Leu Ala Val Asp Phe Pro Ile Phe Pro Arg Arg
145 150 155 160

Phe Ala Lys Val Glu Thr Trp Gly Thr Ser Leu Met Asp Leu Gly Val
165 170 175

Gly Ser Phe Val Phe Ser Asn Gly Ile Val Ser Ser Arg Ala Leu Leu
180 185 190

Lys Asn Leu Ser Leu Lys Ser Lys Pro Ser Phe Leu Lys Asn Ala Phe
195 200 205

Asn Ala Leu Lys Ser Gly Gly Thr Leu Leu Phe Leu Gly Leu Leu Arg
210 215 220

Leu Phe Phe Val Lys Asn Leu Glu Tyr Gln Glu His Val Thr Glu Tyr
225 230 235 240

Gly Val His Trp Asn Phe Phe Ile Thr Leu Ser Leu Leu Pro Leu Val
245 250 255

Leu Thr Phe Ile Asp Pro Val Thr Arg Met Val Pro Arg Cys Ser Ile
260 265 270

Ala Ile Phe Ile Ser Cys Ile Tyr Glu Trp Leu Leu Leu Lys Asp Asp
275 280 285

Arg Thr Leu Asn Phe Leu Ile Leu Ala Asp Arg Asn Cys Phe Phe Ser
290 295 300

Ala Asn Arg Glu Gly Ile Phe Ser Phe Leu Gly Tyr Cys Ser Ile Phe
305 310 315 320

Leu Trp Gly Gln Asn Thr Gly Phe Tyr Leu Leu Gly Asn Lys Pro Thr
325 330 335

Leu Asn Asn Leu Tyr Lys Pro Ser Thr Gln Asp Val Val Ala Ala Ser
340 345 350

Lys Lys Ser Ser Thr Trp Asp Tyr Trp Thr Ser Val Thr Pro Leu Ser
355 360 365

Gly Leu Cys Ile Trp Ser Thr Ile Phe Leu Val Ile Ser Gln Leu Val
370 375 380

Phe Gln Tyr His Pro Tyr Ser Val Ser Arg Arg Phe Ala Asn Leu Pro
385 390 395 400

Tyr Thr Leu Trp Val Ile Thr Tyr Asn Leu Leu Phe Leu Thr Gly Tyr

405 410 415
Cys Leu Thr Asp Lys Ile Phe Gly Asn Ser Ser Glu Tyr Tyr Lys Val
420 425 430
Ala Glu Cys Leu Glu Ser Ile Asn Ser Asn Gly Leu Phe Leu Phe Leu
435 440 445
Leu Ala Asn Val Ser Thr Gly Leu Val Asn Met Ser Met Val Thr Ile
450 455 460
Asp Ser Ser Pro Leu Lys Ser Phe Leu Val Leu Leu Ala Tyr Cys Ser
465 470 475 480
Phe Ile Ala Val Ile Ser Val Phe Leu Tyr Arg Lys Arg Ile Phe Ile
485 490 495
Lys Leu

<210> 3
<211> 1458
<212> DNA
<213> Candida albicans

<220>
<221> CDS
<222> (1)..(1455)

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act ggt ggc aca att gaa gaa att tat gct gta acc agt ata gca tta 96
Thr Gly Gly Thr Ile Glu Glu Ile Tyr Ala Val Thr Ser Ile Ala Leu
20 25 30
tca tct tat ttg tcc ttt aga ttg ttg aaa aag tct ctt ggt gat tta 144
Ser Ser Tyr Leu Ser Phe Arg Leu Leu Lys Lys Ser Leu Gly Asp Leu
35 40 45
gct ttg att tac gac tac att ctt aat gtg ttg aca att cta gca tcc 192
Ala Leu Ile Tyr Asp Tyr Ile Leu Asn Val Leu Thr Ile Leu Ala Ser
50 55 60

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att act gtt tat agc aac agc cct tct tat ttg cat tat ttt att gtt    240
Ile Thr Val Tyr Ser Asn Ser Pro Ser Tyr Leu His Tyr Phe Ile Val
 65                  70                  75                  80
att cca tca tta gtt ata tat cta gtg aat tac cat gtt gag aaa cca    288
Ile Pro Ser Leu Val Ile Tyr Leu Val Asn Tyr His Val Glu Lys Pro
                 85                  90                  95
tct tca ccc cat aga caa aat gat aca aaa gaa gat aaa tcg gac gaa    336
Ser Ser Pro His Arg Gln Asn Asp Thr Lys Glu Asp Lys Ser Asp Glu
            100                 105                 110
cta ttg ccg aga aaa caa ttt ata aca gcc tat cgt tct caa atg ttg    384
Leu Leu Pro Arg Lys Gln Phe Ile Thr Ala Tyr Arg Ser Gln Met Leu
        115                 120                 125
ata att act aat cta gct ata tta gct gtt gat ttt cct att ttc cca    432
Ile Ile Thr Asn Leu Ala Ile Leu Ala Val Asp Phe Pro Ile Phe Pro
    130                 135                 140
aga aga ttt gcc aaa gtg gaa aca tgg ggc acg tca atg atg gat tta    480
Arg Arg Phe Ala Lys Val Glu Thr Trp Gly Thr Ser Met Met Asp Leu
145                 150                 155                 160
gga gtt ggg tcg ttt gtg ttc tcc atg ggg ttg gct aat tct cga caa    528
Gly Val Gly Ser Phe Val Phe Ser Met Gly Leu Ala Asn Ser Arg Gln
                165                 170                 175
ttg atc aag aac cac acc gac aac tac aaa ttt agt tgg aag agt tat    576
Leu Ile Lys Asn His Thr Asp Asn Tyr Lys Phe Ser Trp Lys Ser Tyr
            180                 185                 190
ttg aaa aca atc aag cag aac ttt atc aag tca gtg cct ata ctt gtt    624
Leu Lys Thr Ile Lys Gln Asn Phe Ile Lys Ser Val Pro Ile Leu Val
        195                 200                 205
tta gga gct att cgt ttt gtt agt gtt aag caa ttg gac tat cag gaa    672
Leu Gly Ala Ile Arg Phe Val Ser Val Lys Gln Leu Asp Tyr Gln Glu
    210                 215                 220
cac gaa aca gag tat gga atc cat tgg aat ttt ttc ttc aca tta ggg    720
His Glu Thr Glu Tyr Gly Ile His Trp Asn Phe Phe Phe Thr Leu Gly
225                 230                 235                 240
ttc ttg cca att gta ttg gga ata tta gac ccg gtg ttg aat ttg gtt    768
Phe Leu Pro Ile Val Leu Gly Ile Leu Asp Pro Val Leu Asn Leu Val
                245                 250                 255
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cca cgc ttc ata ata gga att ggt atc tca att gct tat gag gta gcg    816
Pro Arg Phe Ile Ile Gly Ile Gly Ile Ser Ile Ala Tyr Glu Val Ala
            260                 265                 270
ttg aat aag act ggt ttg ttg aag ttc att ttg agc agc gaa aac aga    864
Leu Asn Lys Thr Gly Leu Leu Lys Phe Ile Leu Ser Ser Glu Asn Arg
        275                 280                 285
ctt gaa tct ctc atc acc atg aat aaa gaa ggt att ttt tcg ttt att    912
Leu Glu Ser Leu Ile Thr Met Asn Lys Glu Gly Ile Phe Ser Phe Ile
    290                 295                 300
gga tat ctt tgt att ttt ata att ggt cag tct ttt ggg tca ttt gtt    960
Gly Tyr Leu Cys Ile Phe Ile Ile Gly Gln Ser Phe Gly Ser Phe Val
305                 310                 315                 320
tta aca ggc tac aaa aca aag aac aac tta ata acc att agc aaa att   1008
Leu Thr Gly Tyr Lys Thr Lys Asn Asn Leu Ile Thr Ile Ser Lys Ile
                325                 330                 335
cgt att tca aaa aaa caa cac aag aaa gag ctg ctg ctg ttt ttc tca   1056
Arg Ile Ser Lys Lys Gln His Lys Lys Glu Leu Leu Leu Phe Phe Ser
            340                 345                 350
gtc gcc act act cag gga tta tat ttg gca tgt atc ttc tat cac tta   1104
Val Ala Thr Thr Gln Gly Leu Tyr Leu Ala Cys Ile Phe Tyr His Leu
        355                 360                 365
gct ttc agt ttg ttc atc agc aac tta tca ttc ttg caa cca att tca   1152
Ala Phe Ser Leu Phe Ile Ser Asn Leu Ser Phe Leu Gln Pro Ile Ser
    370                 375                 380
aga cga ttg gcc aat ttc ccc tac gtc atg tgg gtc gtt tcg tac aat   1200
Arg Arg Leu Ala Asn Phe Pro Tyr Val Met Trp Val Val Ser Tyr Asn
385                 390                 395                 400
gct acg ttt tta tta tgt tat gac tta att gaa aaa ttt atc ccg ggg   1248
Ala Thr Phe Leu Leu Cys Tyr Asp Leu Ile Glu Lys Phe Ile Pro Gly
                405                 410                 415
aac ctt act tct act gta ttg gac tct att aat aac aat ggt tta ttt   1296
Asn Leu Thr Ser Thr Val Leu Asp Ser Ile Asn Asn Asn Gly Leu Phe
            420                 425                 430
atc ttc ttg gtc agc aat tta tta aca ggg ttt att aac atg tcc atc   1344
Ile Phe Leu Val Ser Asn Leu Leu Thr Gly Phe Ile Asn Met Ser Ile
        435                 440                 445
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aac act ttg gaa act agc aat aaa atg gca gtg att atc ttg att ggc 1392
Asn Thr Leu Glu Thr Ser Asn Lys Met Ala Val Ile Ile Leu Ile Gly
450 455 460
tat agt ctt act tgg aca ttg ctc gcc tta tat ttg gat aag agg aag 1440
Tyr Ser Leu Thr Trp Thr Leu Leu Ala Leu Tyr Leu Asp Lys Arg Lys
465 470 475 480
atc tac atc aag ctt tag 1458
Ile Tyr Ile Lys Leu
485

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<212> PRT
<213> Candida albicans

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20 25 30
Ser Ser Tyr Leu Ser Phe Arg Leu Leu Lys Lys Ser Leu Gly Asp Leu
35 40 45
Ala Leu Ile Tyr Asp Tyr Ile Leu Asn Val Leu Thr Ile Leu Ala Ser
50 55 60
Ile Thr Val Tyr Ser Asn Ser Pro Ser Tyr Leu His Tyr Phe Ile Val
65 70 75 80
Ile Pro Ser Leu Val Ile Tyr Leu Val Asn Tyr His Val Glu Lys Pro
85 90 95
Ser Ser Pro His Arg Gln Asn Asp Thr Lys Glu Asp Lys Ser Asp Glu
100 105 110
Leu Leu Pro Arg Lys Gln Phe Ile Thr Ala Tyr Arg Ser Gln Met Leu
115 120 125
Ile Ile Thr Asn Leu Ala Ile Leu Ala Val Asp Phe Pro Ile Phe Pro
130 135 140
Arg Arg Phe Ala Lys Val Glu Thr Trp Gly Thr Ser Met Met Asp Leu

145 150 155 160
Gly Val Gly Ser Phe Val Phe Ser Met Gly Leu Ala Asn Ser Arg Gln
165 170 175
Leu Ile Lys Asn His Thr Asp Asn Tyr Lys Phe Ser Trp Lys Ser Tyr
180 185 190
Leu Lys Thr Ile Lys Gln Asn Phe Ile Lys Ser Val Pro Ile Leu Val
195 200 205
Leu Gly Ala Ile Arg Phe Val Ser Val Lys Gln Leu Asp Tyr Gln Glu
210 215 220
His Glu Thr Glu Tyr Gly Ile His Trp Asn Phe Phe Phe Thr Leu Gly
225 230 235 240
Phe Leu Pro Ile Val Leu Gly Ile Leu Asp Pro Val Leu Asn Leu Val
245 250 255
Pro Arg Phe Ile Ile Gly Ile Gly Ile Ser Ile Ala Tyr Glu Val Ala
260 265 270
Leu Asn Lys Thr Gly Leu Leu Lys Phe Ile Leu Ser Ser Glu Asn Arg
275 280 285
Leu Glu Ser Leu Ile Thr Met Asn Lys Glu Gly Ile Phe Ser Phe Ile
290 295 300
Gly Tyr Leu Cys Ile Phe Ile Ile Gly Gln Ser Phe Gly Ser Phe Val
305 310 315 320
Leu Thr Gly Tyr Lys Thr Lys Asn Asn Leu Ile Thr Ile Ser Lys Ile
325 330 335
Arg Ile Ser Lys Lys Gln His Lys Lys Glu Leu Leu Leu Phe Phe Ser
340 345 350
Val Ala Thr Thr Gln Gly Leu Tyr Leu Ala Cys Ile Phe Tyr His Leu
355 360 365
Ala Phe Ser Leu Phe Ile Ser Asn Leu Ser Phe Leu Gln Pro Ile Ser
370 375 380
Arg Arg Leu Ala Asn Phe Pro Tyr Val Met Trp Val Val Ser Tyr Asn
385 390 395 400
Ala Thr Phe Leu Leu Cys Tyr Asp Leu Ile Glu Lys Phe Ile Pro Gly
405 410 415
Asn Leu Thr Ser Thr Val Leu Asp Ser Ile Asn Asn Asn Gly Leu Phe
420 425 430
Ile Phe Leu Val Ser Asn Leu Leu Thr Gly Phe Ile Asn Met Ser Ile

435 440 445

Asn Thr Leu Glu Thr Ser Asn Lys Met Ala Val Ile Ile Leu Ile Gly

450 455 460

Tyr Ser Leu Thr Trp Thr Leu Leu Ala Leu Tyr Leu Asp Lys Arg Lys

465 470 475 480

Ile Tyr Ile Lys Leu

485

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<211> 1458

<212> DNA

<213> Candida albicans

<220>

<221> CDS

<222> (1)..(1455)

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1 5 10 15

act ggt ggc aca att gaa gaa att tat gct gta acc agt ata gca tta 96

Thr Gly Gly Thr Ile Glu Glu Ile Tyr Ala Val Thr Ser Ile Ala Leu

20 25 30

tca tct tat ttg tcc ttt aga ttg ttg aaa aag tct ctt ggt gat tta 144

Ser Ser Tyr Leu Ser Phe Arg Leu Leu Lys Lys Ser Leu Gly Asp Leu

35 40 45

gct ttg att tac gac tac att ctt aat gtg ttg aca att cta gca tcc 192

Ala Leu Ile Tyr Asp Tyr Ile Leu Asn Val Leu Thr Ile Leu Ala Ser

50 55 60

att act gtt tat agc aac agc cct tct tat ttg cat tat ttt att gtt 240

Ile Thr Val Tyr Ser Asn Ser Pro Ser Tyr Leu His Tyr Phe Ile Val

65 70 75 80

att cca tca tta gtt ata tat cta gtg aat tac cat gtt gag aaa cca 288

Ile Pro Ser Leu Val Ile Tyr Leu Val Asn Tyr His Val Glu Lys Pro

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                    85                  90                  95
tct tca ccc cat aga caa aat gat aca aaa gaa gat aaa tcg gac gaa   336
Ser Ser Pro His Arg Gln Asn Asp Thr Lys Glu Asp Lys Ser Asp Glu
                100                 105                 110
cta ttg ccg aga aaa caa ttt ata aca gcc tat cgt tct caa atg ttg   384
Leu Leu Pro Arg Lys Gln Phe Ile Thr Ala Tyr Arg Ser Gln Met Leu
        115                 120                 125
ata att act aat cta gct ata tta gct gtt gat ttt cct att ttc cca   432
Ile Ile Thr Asn Leu Ala Ile Leu Ala Val Asp Phe Pro Ile Phe Pro
    130                 135                 140
aga aga ttt gcc aaa gtg gaa aca tgg ggc acg tca atg atg gat tta   480
Arg Arg Phe Ala Lys Val Glu Thr Trp Gly Thr Ser Met Met Asp Leu
145                 150                 155                 160
gga gtt ggg tcg ttt gtg ttc tcc atg ggg ttg gct aat tct cga caa   528
Gly Val Gly Ser Phe Val Phe Ser Met Gly Leu Ala Asn Ser Arg Gln
                165                 170                 175
ttg atc aag aac cac acc gac aat tac aaa ttt agt tgg aag agt tat   576
Leu Ile Lys Asn His Thr Asp Asn Tyr Lys Phe Ser Trp Lys Ser Tyr
            180                 185                 190
ttg aaa aca atc aag cag aac ttt atc aag tca gtg cct ata ctt gtt   624
Leu Lys Thr Ile Lys Gln Asn Phe Ile Lys Ser Val Pro Ile Leu Val
        195                 200                 205
tta gga gct att cgt ttt gtt agt gtt aag caa ttg gac tat cag gaa   672
Leu Gly Ala Ile Arg Phe Val Ser Val Lys Gln Leu Asp Tyr Gln Glu
    210                 215                 220
cac gaa aca gag tat gga atc cat tgg aat ttt ttc ttc aca tta ggg   720
His Glu Thr Glu Tyr Gly Ile His Trp Asn Phe Phe Phe Thr Leu Gly
225                 230                 235                 240
ttc ttg cca att gta ttg gga ata tta gac ccg gtg ttg aat ttg gtt   768
Phe Leu Pro Ile Val Leu Gly Ile Leu Asp Pro Val Leu Asn Leu Val
                245                 250                 255
cca cgc ttc ata ata gga att ggt atc tca att ggt tat gag gta gcg   816
Pro Arg Phe Ile Ile Gly Ile Gly Ile Ser Ile Gly Tyr Glu Val Ala
            260                 265                 270
ttg aat aag act ggt ttg ttg aag ttc att ttg agc agc gaa aac aga   864
Leu Asn Lys Thr Gly Leu Leu Lys Phe Ile Leu Ser Ser Glu Asn Arg
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                275                 280                 285
ctt gaa tct ctc atc gcc atg aat aaa gaa ggt att ttt tcg ttt att    912
Leu Glu Ser Leu Ile Ala Met Asn Lys Glu Gly Ile Phe Ser Phe Ile
    290                 295                 300
gga tat ctt tgt att ttt ata att ggt cag tct ttt ggg tca ttt gtt    960
Gly Tyr Leu Cys Ile Phe Ile Ile Gly Gln Ser Phe Gly Ser Phe Val
305                 310                 315                 320
tta aca ggc tac aaa aca aag aac aac tta ata acc att agc aaa att   1008
Leu Thr Gly Tyr Lys Thr Lys Asn Asn Leu Ile Thr Ile Ser Lys Ile
                325                 330                 335
cgt att tca aaa aaa caa cac aag aaa gag ctg ctg ctg ttt ttc tca   1056
Arg Ile Ser Lys Lys Gln His Lys Lys Glu Leu Leu Leu Phe Phe Ser
            340                 345                 350
gtc gcc act act cag gga tta tat ttg gca tgt atc ttc tat cac tta   1104
Val Ala Thr Thr Gln Gly Leu Tyr Leu Ala Cys Ile Phe Tyr His Leu
        355                 360                 365
gct ttc agt ttg ttc atc agc aac tta tca ttc ttg caa cca att tca   1152
Ala Phe Ser Leu Phe Ile Ser Asn Leu Ser Phe Leu Gln Pro Ile Ser
    370                 375                 380
aga cga ttg gcc aat ttc ccc tac gtc atg tgg gtc gtt tcg tac aat   1200
Arg Arg Leu Ala Asn Phe Pro Tyr Val Met Trp Val Val Ser Tyr Asn
385                 390                 395                 400
gct acg ttt tta tta tgt tat gac tta att gaa aaa ttt atc ccg ggg   1248
Ala Thr Phe Leu Leu Cys Tyr Asp Leu Ile Glu Lys Phe Ile Pro Gly
                405                 410                 415
aac ctt act tct act gta ttg gac tct att aat aac aat ggt tta ttt   1296
Asn Leu Thr Ser Thr Val Leu Asp Ser Ile Asn Asn Asn Gly Leu Phe
            420                 425                 430
atc ttc ttg gtc agc aat tta tta aca ggg ttt att aac atg tcc atc   1344
Ile Phe Leu Val Ser Asn Leu Leu Thr Gly Phe Ile Asn Met Ser Ile
        435                 440                 445
aac act ttg gaa act agc aat aaa atg gca gtg att atc ttg att ggc   1392
Asn Thr Leu Glu Thr Ser Asn Lys Met Ala Val Ile Ile Leu Ile Gly
    450                 455                 460
tat agt ctt act tgg aca ttg ctc gcc tta tat ttg gat aag agg aag   1440
Tyr Ser Leu Thr Trp Thr Leu Leu Ala Leu Tyr Leu Asp Lys Arg Lys
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465 470 475 480

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Ile Tyr Ile Lys Leu
485

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<211> 485
<212> PRT
<213> Candida albicans

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20 25 30
Ser Ser Tyr Leu Ser Phe Arg Leu Leu Lys Lys Ser Leu Gly Asp Leu
35 40 45
Ala Leu Ile Tyr Asp Tyr Ile Leu Asn Val Leu Thr Ile Leu Ala Ser
50 55 60
Ile Thr Val Tyr Ser Asn Ser Pro Ser Tyr Leu His Tyr Phe Ile Val
65 70 75 80
Ile Pro Ser Leu Val Ile Tyr Leu Val Asn Tyr His Val Glu Lys Pro
85 90 95
Ser Ser Pro His Arg Gln Asn Asp Thr Lys Glu Asp Lys Ser Asp Glu
100 105 110
Leu Leu Pro Arg Lys Gln Phe Ile Thr Ala Tyr Arg Ser Gln Met Leu
115 120 125
Ile Ile Thr Asn Leu Ala Ile Leu Ala Val Asp Phe Pro Ile Phe Pro
130 135 140
Arg Arg Phe Ala Lys Val Glu Thr Trp Gly Thr Ser Met Met Asp Leu
145 150 155 160
Gly Val Gly Ser Phe Val Phe Ser Met Gly Leu Ala Asn Ser Arg Gln
165 170 175
Leu Ile Lys Asn His Thr Asp Asn Tyr Lys Phe Ser Trp Lys Ser Tyr
180 185 190

Leu Lys Thr Ile Lys Gln Asn Phe Ile Lys Ser Val Pro Ile Leu Val
195 200 205
Leu Gly Ala Ile Arg Phe Val Ser Val Lys Gln Leu Asp Tyr Gln Glu
210 215 220
His Glu Thr Glu Tyr Gly Ile His Trp Asn Phe Phe Phe Thr Leu Gly
225 230 235 240
Phe Leu Pro Ile Val Leu Gly Ile Leu Asp Pro Val Leu Asn Leu Val
245 250 255
Pro Arg Phe Ile Ile Gly Ile Gly Ile Ser Ile Gly Tyr Glu Val Ala
260 265 270
Leu Asn Lys Thr Gly Leu Leu Lys Phe Ile Leu Ser Ser Glu Asn Arg
275 280 285
Leu Glu Ser Leu Ile Ala Met Asn Lys Glu Gly Ile Phe Ser Phe Ile
290 295 300
Gly Tyr Leu Cys Ile Phe Ile Ile Gly Gln Ser Phe Gly Ser Phe Val
305 310 315 320
Leu Thr Gly Tyr Lys Thr Lys Asn Asn Leu Ile Thr Ile Ser Lys Ile
325 330 335
Arg Ile Ser Lys Lys Gln His Lys Lys Glu Leu Leu Leu Phe Phe Ser
340 345 350
Val Ala Thr Thr Gln Gly Leu Tyr Leu Ala Cys Ile Phe Tyr His Leu
355 360 365
Ala Phe Ser Leu Phe Ile Ser Asn Leu Ser Phe Leu Gln Pro Ile Ser
370 375 380
Arg Arg Leu Ala Asn Phe Pro Tyr Val Met Trp Val Val Ser Tyr Asn
385 390 395 400
Ala Thr Phe Leu Leu Cys Tyr Asp Leu Ile Glu Lys Phe Ile Pro Gly
405 410 415
Asn Leu Thr Ser Thr Val Leu Asp Ser Ile Asn Asn Asn Gly Leu Phe
420 425 430
Ile Phe Leu Val Ser Asn Leu Leu Thr Gly Phe Ile Asn Met Ser Ile
435 440 445
Asn Thr Leu Glu Thr Ser Asn Lys Met Ala Val Ile Ile Leu Ile Gly
450 455 460
Tyr Ser Leu Thr Trp Thr Leu Leu Ala Leu Tyr Leu Asp Lys Arg Lys
465 470 475 480

Ile Tyr Ile Lys Leu
485

<210> 7
<211> 1380
<212> DNA
<213> Schizosaccharomyces pombe

<220>
<221> CDS
<222> (1)..(1380)

<400> 7

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Met Ser Tyr Lys Leu Glu Lys Glu Ala Phe Val Ser Asn Leu Thr Gly
  1               5                  10                  15
tca agt tcc att gag aca tgt ggc ttg tta tta ata gga att gct tgc     96
Ser Ser Ser Ile Glu Thr Cys Gly Leu Leu Leu Ile Gly Ile Ala Cys
             20                  25                  30
aac gtt ttg tgg gta aac atg act gcg aga aac atc tta ccc aaa ggg    144
Asn Val Leu Trp Val Asn Met Thr Ala Arg Asn Ile Leu Pro Lys Gly
         35                  40                  45
aat ctt ggg ttt ctt gtt gag ttt ttc atc ttt tgc tta att cca tta    192
Asn Leu Gly Phe Leu Val Glu Phe Phe Ile Phe Cys Leu Ile Pro Leu
     50                  55                  60
ttt gtc att tac gtt tca tcg aaa gtt ggc gtt ttc act ctt tgc ata    240
Phe Val Ile Tyr Val Ser Ser Lys Val Gly Val Phe Thr Leu Cys Ile
 65                  70                  75                  80
gcc tct ttt ttg cct tcc ttc gtc ctt cat gtt ata agt cca att aat    288
Ala Ser Phe Leu Pro Ser Phe Val Leu His Val Ile Ser Pro Ile Asn
                 85                  90                  95
tgg gat gtg ctg aga aga aaa cct ggt tgt tgt ctt act aaa aaa aat    336
Trp Asp Val Leu Arg Arg Lys Pro Gly Cys Cys Leu Thr Lys Lys Asn
            100                 105                 110
gaa aat act ttt gat cga cga att gct gga gtc aca ttt tat cgt tct    384
Glu Asn Thr Phe Asp Arg Arg Ile Ala Gly Val Thr Phe Tyr Arg Ser
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                115                 120                 125
caa atg atg ttg gtt act gtc act tgc atc ctg gcc gtt gac ttt acc     432
Gln Met Met Leu Val Thr Val Thr Cys Ile Leu Ala Val Asp Phe Thr
    130                 135                 140
ctt ttc ccg agg aga tat gcc aaa gtt gaa acc tgg gga aca tca ctg     480
Leu Phe Pro Arg Arg Tyr Ala Lys Val Glu Thr Trp Gly Thr Ser Leu
145                 150                 155                 160
atg gat ctt ggt gtt gga tct ttc atg ttt tct tca ggt act gtg gct     528
Met Asp Leu Gly Val Gly Ser Phe Met Phe Ser Ser Gly Thr Val Ala
                165                 170                 175
gga cgg aaa aat gac att aaa aaa cca aat gcg ttt aaa aat gta ttg     576
Gly Arg Lys Asn Asp Ile Lys Lys Pro Asn Ala Phe Lys Asn Val Leu
            180                 185                 190
tgg aat tct ttc atc ctt ttg att tta gga ttt gcg cgc atg ttt tta     624
Trp Asn Ser Phe Ile Leu Leu Ile Leu Gly Phe Ala Arg Met Phe Leu
        195                 200                 205
acg aaa agc atc aat tac caa gaa cat gta agc gaa tat ggc atg cat     672
Thr Lys Ser Ile Asn Tyr Gln Glu His Val Ser Glu Tyr Gly Met His
    210                 215                 220
tgg aac ttt ttt ttc acc cta ggt ttc atg gct ctt ggc gta ttt ttt     720
Trp Asn Phe Phe Phe Thr Leu Gly Phe Met Ala Leu Gly Val Phe Phe
225                 230                 235                 240
ttt cgt cgt tct tta aaa aaa gtc tcc tat ttt aat tta gca acc ttc     768
Phe Arg Arg Ser Leu Lys Lys Val Ser Tyr Phe Asn Leu Ala Thr Phe
                245                 250                 255
att act ctt ctt cat cat tgt ttg ctt gtt tta acc cct ttc caa aaa     816
Ile Thr Leu Leu His His Cys Leu Leu Val Leu Thr Pro Phe Gln Lys
            260                 265                 270
tgg gca cta tcc gcc ccc aga aca aat att ttg gct cag aat aga gag     864
Trp Ala Leu Ser Ala Pro Arg Thr Asn Ile Leu Ala Gln Asn Arg Glu
        275                 280                 285
ggt att gct tct ctt ccc gga tac att gct att tac ttt tat gga atg     912
Gly Ile Ala Ser Leu Pro Gly Tyr Ile Ala Ile Tyr Phe Tyr Gly Met
    290                 295                 300
tat acc ggt agt gta gtt ttg gct gat cga cct cta atg tat act aga     960
Tyr Thr Gly Ser Val Val Leu Ala Asp Arg Pro Leu Met Tyr Thr Arg
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305                 310                 315                 320
gct gag tcg tgg aag cgc ttt caa cgt cta tta ttc ccg cta tgc att   1008
Ala Glu Ser Trp Lys Arg Phe Gln Arg Leu Leu Phe Pro Leu Cys Ile
                325                 330                 335
ttg tta gtg ttg tat ctt gtg tct aac ttt ttg tca gtt ggt gtt tct   1056
Leu Leu Val Leu Tyr Leu Val Ser Asn Phe Leu Ser Val Gly Val Ser
            340                 345                 350
cgc cga ctt gct aat acg cct tat gtt gcg aat gtt gcc ttt atc aat   1104
Arg Arg Leu Ala Asn Thr Pro Tyr Val Ala Asn Val Ala Phe Ile Asn
        355                 360                 365
atg ttt ttt ctt act ata tac ata ctt att gat gcc tat tta ttc cca   1152
Met Phe Phe Leu Thr Ile Tyr Ile Leu Ile Asp Ala Tyr Leu Phe Pro
    370                 375                 380
tct tct gtg cca tat gga agt cgc gtc ccc aaa ctg ctt gaa gat gcc   1200
Ser Ser Val Pro Tyr Gly Ser Arg Val Pro Lys Leu Leu Glu Asp Ala
385                 390                 395                 400
aat aat aat ggc ttg ttg gtg ttt ttg att gct aac gtt tta aca gga   1248
Asn Asn Asn Gly Leu Leu Val Phe Leu Ile Ala Asn Val Leu Thr Gly
                405                 410                 415
gta gtt aat tta tcg ttc gac acc ctt cat tct agc aat gca aaa ggc   1296
Val Val Asn Leu Ser Phe Asp Thr Leu His Ser Ser Asn Ala Lys Gly
            420                 425                 430
ttg aca atc atg act atg tat ctt ttt att att tgc tat atg gca cat   1344
Leu Thr Ile Met Thr Met Tyr Leu Phe Ile Ile Cys Tyr Met Ala His
        435                 440                 445
tgg ctt gct caa cac gga att cgt ttt cgc ctt tag                  1380
Trp Leu Ala Gln His Gly Ile Arg Phe Arg Leu
    450                 455                 460
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<211> 459
<212> PRT
<213> Schizosaccharomyces pombe

<400> 8

Met Ser Tyr Lys Leu Glu Lys Glu Ala Phe Val Ser Asn Leu Thr Gly
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Ser Ser Ser Ile Glu Thr Cys Gly Leu Leu Leu Ile Gly Ile Ala Cys
20 25 30
Asn Val Leu Trp Val Asn Met Thr Ala Arg Asn Ile Leu Pro Lys Gly
35 40 45
Asn Leu Gly Phe Leu Val Glu Phe Phe Ile Phe Cys Leu Ile Pro Leu
50 55 60
Phe Val Ile Tyr Val Ser Ser Lys Val Gly Val Phe Thr Leu Cys Ile
65 70 75 80
Ala Ser Phe Leu Pro Ser Phe Val Leu His Val Ile Ser Pro Ile Asn
85 90 95
Trp Asp Val Leu Arg Arg Lys Pro Gly Cys Cys Leu Thr Lys Lys Asn
100 105 110
Glu Asn Thr Phe Asp Arg Arg Ile Ala Gly Val Thr Phe Tyr Arg Ser
115 120 125
Gln Met Met Leu Val Thr Val Thr Cys Ile Leu Ala Val Asp Phe Thr
130 135 140
Leu Phe Pro Arg Arg Tyr Ala Lys Val Glu Thr Trp Gly Thr Ser Leu
145 150 155 160
Met Asp Leu Gly Val Gly Ser Phe Met Phe Ser Ser Gly Thr Val Ala
165 170 175
Gly Arg Lys Asn Asp Ile Lys Lys Pro Asn Ala Phe Lys Asn Val Leu
180 185 190
Trp Asn Ser Phe Ile Leu Leu Ile Leu Gly Phe Ala Arg Met Phe Leu
195 200 205
Thr Lys Ser Ile Asn Tyr Gln Glu His Val Ser Glu Tyr Gly Met His
210 215 220
Trp Asn Phe Phe Phe Thr Leu Gly Phe Met Ala Leu Gly Val Phe Phe
225 230 235 240
Phe Arg Arg Ser Leu Lys Lys Val Ser Tyr Phe Asn Leu Ala Thr Phe
245 250 255
Ile Thr Leu Leu His His Cys Leu Leu Val Leu Thr Pro Phe Gln Lys
260 265 270
Trp Ala Leu Ser Ala Pro Arg Thr Asn Ile Leu Ala Gln Asn Arg Glu
275 280 285

Gly Ile Ala Ser Leu Pro Gly Tyr Ile Ala Ile Tyr Phe Tyr Gly Met
290 295 300
Tyr Thr Gly Ser Val Val Leu Ala Asp Arg Pro Leu Met Tyr Thr Arg
305 310 315 320
Ala Glu Ser Trp Lys Arg Phe Gln Arg Leu Leu Phe Pro Leu Cys Ile
325 330 335
Leu Leu Val Leu Tyr Leu Val Ser Asn Phe Leu Ser Val Gly Val Ser
340 345 350
Arg Arg Leu Ala Asn Thr Pro Tyr Val Ala Asn Val Ala Phe Ile Asn
355 360 365
Met Phe Phe Leu Thr Ile Tyr Ile Leu Ile Asp Ala Tyr Leu Phe Pro
370 375 380
Ser Ser Val Pro Tyr Gly Ser Arg Val Pro Lys Leu Leu Glu Asp Ala
385 390 395 400
Asn Asn Asn Gly Leu Leu Val Phe Leu Ile Ala Asn Val Leu Thr Gly
405 410 415
Val Val Asn Leu Ser Phe Asp Thr Leu His Ser Ser Asn Ala Lys Gly
420 425 430
Leu Thr Ile Met Thr Met Tyr Leu Phe Ile Ile Cys Tyr Met Ala His
435 440 445
Trp Leu Ala Gln His Gly Ile Arg Phe Arg Leu
450 455

<210> 9
<211> 1576
<212> DNA
<213> Aspergillus fumigatus

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<222> (31)..(1536)

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Met Asp Pro Asp Tyr Lys Ala Arg

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                                                  1               5
    aaa gag gcc ttt gtc tca ggt ctt gca gga gga agc atc ctg gaa atc    102
    Lys Glu Ala Phe Val Ser Gly Leu Ala Gly Gly Ser Ile Leu Glu Ile
         10                  15                  20
    aac gcc gtc acc ttg gtt gct tcg gta tcc gtt ttt ctg tgg tca att    150
    Asn Ala Val Thr Leu Val Ala Ser Val Ser Val Phe Leu Trp Ser Ile
     25                  30                  35                  40
    cta caa tct cgc cta tcc ttt ttc aca ccc tac agc gcc gct gcc ctt    198
    Leu Gln Ser Arg Leu Ser Phe Phe Thr Pro Tyr Ser Ala Ala Ala Leu
                 45                  50                  55
    ctc gtt gat ttc ctg ctc aat gta cta gct atc ttg ttc gca acc act    246
    Leu Val Asp Phe Leu Leu Asn Val Leu Ala Ile Leu Phe Ala Thr Thr
                 60                  65                  70
    tta tac tct tcg gcg cct ctt ctt ctc aat ctc ctt cta ata tct ccc    294
    Leu Tyr Ser Ser Ala Pro Leu Leu Leu Asn Leu Leu Leu Ile Ser Pro
             75                  80                  85
    gct ctg ctg ata ctc ctc tct acg aaa cgt cct cgg acc ccc gtc aaa    342
    Ala Leu Leu Ile Leu Leu Ser Thr Lys Arg Pro Arg Thr Pro Val Lys
         90                  95                  100
    gcg aaa cct cct cgc cag tcc gct aga gct ggg aaa gat gac tcg aaa    390
    Ala Lys Pro Pro Arg Gln Ser Ala Arg Ala Gly Lys Asp Asp Ser Lys
    105                 110                 115                 120
    cat gcg aca gcc ttg cca gag tct cta ccc att cat cca ttt ctc acg    438
    His Ala Thr Ala Leu Pro Glu Ser Leu Pro Ile His Pro Phe Leu Thr
                125                 130                 135
    aca tat cgc gcc gcc atg atg gtt atc acg tgc atc gct atc ttg gct    486
    Thr Tyr Arg Ala Ala Met Met Val Ile Thr Cys Ile Ala Ile Leu Ala
                140                 145                 150
    gtg gat ttt cgc att ttt cct cgc cga ttc gcc aag gta gaa aac tgg    534
    Val Asp Phe Arg Ile Phe Pro Arg Arg Phe Ala Lys Val Glu Asn Trp
            155                 160                 165
    ggt aca tca ctc atg gat ctg ggc gtt gga tcg ttt gtc ttt tcg ggc    582
    Gly Thr Ser Leu Met Asp Leu Gly Val Gly Ser Phe Val Phe Ser Gly
        170                 175                 180
    gga gta gta tcc gct cgc tca cta ctc aag agc agg acc aat ggc tct    630
    Gly Val Val Ser Ala Arg Ser Leu Leu Lys Ser Arg Thr Asn Gly Ser
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185 190 195 200

aaa agg ttg cct ctt gcc aag agg ttg att gcg tcg acg cga cac tct 678
Lys Arg Leu Pro Leu Ala Lys Arg Leu Ile Ala Ser Thr Arg His Ser
205 210 215

att cct ctg ctc gtc ctc ggc ctg att cgg cta tac agc gtc aaa ggc 726
Ile Pro Leu Leu Val Leu Gly Leu Ile Arg Leu Tyr Ser Val Lys Gly
220 225 230

ttg gac tat gcg gag cac gtc acc gag tac ggc gta cat tgg aac ttc 774
Leu Asp Tyr Ala Glu His Val Thr Glu Tyr Gly Val His Trp Asn Phe
235 240 245

ttc ttt aca ttg ggt ctt ttg cct ccg ttc gtg gag gtc ttc gac gcc 822
Phe Phe Thr Leu Gly Leu Leu Pro Pro Phe Val Glu Val Phe Asp Ala
250 255 260

ttg gct acg atc att ccg tca tac gag gtt ctc tcc gtg ggg atc gcc 870
Leu Ala Thr Ile Ile Pro Ser Tyr Glu Val Leu Ser Val Gly Ile Ala
265 270 275 280

gtc ttg tat caa gtt gcc cta gag tca aca gac ttg aaa agc tac atc 918
Val Leu Tyr Gln Val Ala Leu Glu Ser Thr Asp Leu Lys Ser Tyr Ile
285 290 295

ctc gtc tcc cct cgt ggg cca agc tta ctg tcc aag aat cgt gaa ggc 966
Leu Val Ser Pro Arg Gly Pro Ser Leu Leu Ser Lys Asn Arg Glu Gly
300 305 310

gtc ttc tcc ttc tca ggt tat ctc gcg att ttt ctt gct ggt cgt gcg 1014
Val Phe Ser Phe Ser Gly Tyr Leu Ala Ile Phe Leu Ala Gly Arg Ala
315 320 325

atc ggc att cgg ata atc cct cgc gga act tct ttc tca aga agc cca 1062
Ile Gly Ile Arg Ile Ile Pro Arg Gly Thr Ser Phe Ser Arg Ser Pro
330 335 340

gaa cag gcc agg aga cgg gtc ctg atc agc ctt ggc gtg caa gcg tta 1110
Glu Gln Ala Arg Arg Arg Val Leu Ile Ser Leu Gly Val Gln Ala Leu
345 350 355 360

gtg tgg acc act ctt ttt gtg ttg aac tcc act tat gcg atg gga tac 1158
Val Trp Thr Thr Leu Phe Val Leu Asn Ser Thr Tyr Ala Met Gly Tyr
365 370 375

gga gct aat atc cct gtc tcc cgc cgc ctc gct aac atg ccc tat gtc 1206
Gly Ala Asn Ile Pro Val Ser Arg Arg Leu Ala Asn Met Pro Tyr Val

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                380                 385                 390
ctt tgg gtt tcg gcg ttc aac acc gcg caa ctg ttt gtg ttc tgc ctg   1254
Leu Trp Val Ser Ala Phe Asn Thr Ala Gln Leu Phe Val Phe Cys Leu
        395                 400                 405
atc gaa aca ctc tgc ttt cct gca gtt cat cgg aca acg act caa gag   1302
Ile Glu Thr Leu Cys Phe Pro Ala Val His Arg Thr Thr Thr Gln Glu
    410                 415                 420
agc gaa tct gag cga gtc gat ttt gct acg agc cga atc atg tcg gcc   1350
Ser Glu Ser Glu Arg Val Asp Phe Ala Thr Ser Arg Ile Met Ser Ala
425                 430                 435                 440
ttc aat aag aac agt ctc gcg atc ttt ctt ttg gcc aat ctt ctg act   1398
Phe Asn Lys Asn Ser Leu Ala Ile Phe Leu Leu Ala Asn Leu Leu Thr
                445                 450                 455
gga gct gtg aat ctg agc atc tcc aca att gat gct aat aca gcg cag   1446
Gly Ala Val Asn Leu Ser Ile Ser Thr Ile Asp Ala Asn Thr Ala Gln
            460                 465                 470
gcc atc gct gtt ctc att gga tat tca tcc att atc aca ggg gtt gct   1494
Ala Ile Ala Val Leu Ile Gly Tyr Ser Ser Ile Ile Thr Gly Val Ala
        475                 480                 485
cta gca ttg cat cat gcc aat atc aaa gta ctt cct ttc tag           1536
Leu Ala Leu His His Ala Asn Ile Lys Val Leu Pro Phe
    490                 495                 500
ggtatttacg agcaattggt ggtgtgttga agatatatag                       1576
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<210> 10
<211> 501
<212> PRT
<213> Aspergillus fumigatus

<400> 10

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Met Asp Pro Asp Tyr Lys Ala Arg Lys Glu Ala Phe Val Ser Gly Leu
  1               5                  10                  15
Ala Gly Gly Ser Ile Leu Glu Ile Asn Ala Val Thr Leu Val Ala Ser
             20                  25                  30
Val Ser Val Phe Leu Trp Ser Ile Leu Gln Ser Arg Leu Ser Phe Phe
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35 40 45

Thr Pro Tyr Ser Ala Ala Ala Leu Leu Val Asp Phe Leu Leu Asn Val
50 55 60

Leu Ala Ile Leu Phe Ala Thr Thr Leu Tyr Ser Ser Ala Pro Leu Leu
65 70 75 80

Leu Asn Leu Leu Leu Ile Ser Pro Ala Leu Leu Ile Leu Leu Ser Thr
85 90 95

Lys Arg Pro Arg Thr Pro Val Lys Ala Lys Pro Pro Arg Gln Ser Ala
100 105 110

Arg Ala Gly Lys Asp Asp Ser Lys His Ala Thr Ala Leu Pro Glu Ser
115 120 125

Leu Pro Ile His Pro Phe Leu Thr Thr Tyr Arg Ala Ala Met Met Val
130 135 140

Ile Thr Cys Ile Ala Ile Leu Ala Val Asp Phe Arg Ile Phe Pro Arg
145 150 155 160

Arg Phe Ala Lys Val Glu Asn Trp Gly Thr Ser Leu Met Asp Leu Gly
165 170 175

Val Gly Ser Phe Val Phe Ser Gly Gly Val Val Ser Ala Arg Ser Leu
180 185 190

Leu Lys Ser Arg Thr Asn Gly Ser Lys Arg Leu Pro Leu Ala Lys Arg
195 200 205

Leu Ile Ala Ser Thr Arg His Ser Ile Pro Leu Leu Val Leu Gly Leu
210 215 220

Ile Arg Leu Tyr Ser Val Lys Gly Leu Asp Tyr Ala Glu His Val Thr
225 230 235 240

Glu Tyr Gly Val His Trp Asn Phe Phe Phe Thr Leu Gly Leu Leu Pro
245 250 255

Pro Phe Val Glu Val Phe Asp Ala Leu Ala Thr Ile Ile Pro Ser Tyr
260 265 270

Glu Val Leu Ser Val Gly Ile Ala Val Leu Tyr Gln Val Ala Leu Glu
275 280 285

Ser Thr Asp Leu Lys Ser Tyr Ile Leu Val Ser Pro Arg Gly Pro Ser
290 295 300

Leu Leu Ser Lys Asn Arg Glu Gly Val Phe Ser Phe Ser Gly Tyr Leu
305 310 315 320

Ala Ile Phe Leu Ala Gly Arg Ala Ile Gly Ile Arg Ile Ile Pro Arg

325 330 335
Gly Thr Ser Phe Ser Arg Ser Pro Glu Gln Ala Arg Arg Arg Val Leu
340 345 350
Ile Ser Leu Gly Val Gln Ala Leu Val Trp Thr Thr Leu Phe Val Leu
355 360 365
Asn Ser Thr Tyr Ala Met Gly Tyr Gly Ala Asn Ile Pro Val Ser Arg
370 375 380
Arg Leu Ala Asn Met Pro Tyr Val Leu Trp Val Ser Ala Phe Asn Thr
385 390 395 400
Ala Gln Leu Phe Val Phe Cys Leu Ile Glu Thr Leu Cys Phe Pro Ala
405 410 415
Val His Arg Thr Thr Thr Gln Glu Ser Glu Ser Glu Arg Val Asp Phe
420 425 430
Ala Thr Ser Arg Ile Met Ser Ala Phe Asn Lys Asn Ser Leu Ala Ile
435 440 445
Phe Leu Leu Ala Asn Leu Leu Thr Gly Ala Val Asn Leu Ser Ile Ser
450 455 460
Thr Ile Asp Ala Asn Thr Ala Gln Ala Ile Ala Val Leu Ile Gly Tyr
465 470 475 480
Ser Ser Ile Ile Thr Gly Val Ala Leu Ala Leu His His Ala Asn Ile
485 490 495
Lys Val Leu Pro Phe
500

<210> 11
<211> 1648
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<213> Aspergillus fumigatus

<220>
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<222> (122)..(198)

<220>
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<222> (26)..(121)

<220>
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<222> (199)..(1608)

<400> 11

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                            Met Asp Pro Asp Tyr Lys Ala Arg Lys
                              1               5

gag gcc ttt gtc tca ggt ctt gca gga gga agc atc ctg gaa atc aac   100
Glu Ala Phe Val Ser Gly Leu Ala Gly Gly Ser Ile Leu Glu Ile Asn
 10                  15                  20                  25

gcc gtc acc ttg gtt gct tcg gttcgtgtta ctatcttatt gtggctactt      151
Ala Val Thr Leu Val Ala Ser
                 30

cgcctacatt gtttctcgac taaccgagtc tctttgcgat caatcag gta tcc gtt   207
                                                 Val Ser Val
                                                      35

ttt ctg tgg tca att cta caa tct cgc cta tcc ttt ttc aca ccc tac   255
Phe Leu Trp Ser Ile Leu Gln Ser Arg Leu Ser Phe Phe Thr Pro Tyr
                 40                  45                  50

agc gcc gct gcc ctt ctc gtt gat ttc ctg ctc aat gta cta gct atc   303
Ser Ala Ala Ala Leu Leu Val Asp Phe Leu Leu Asn Val Leu Ala Ile
             55                  60                  65

ttg ttc gca acc act tta tac tct tcg gcg cct ctt ctt ctc aat ctc   351
Leu Phe Ala Thr Thr Leu Tyr Ser Ser Ala Pro Leu Leu Leu Asn Leu
         70                  75                  80

ctt cta ata tct ccc gct ctg ctg ata ctc ctc tct acg aaa cgt cct   399
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Leu Leu Ile Ser Pro Ala Leu Leu Ile Leu Leu Ser Thr Lys Arg Pro
     85                  90                  95

cgg acc ccc gtc aaa gcg aaa cct cct cgc cag tcc gct aga gct ggg   447
Arg Thr Pro Val Lys Ala Lys Pro Pro Arg Gln Ser Ala Arg Ala Gly
100                 105                 110                 115

aaa gat gac tcg aaa cat gcg aca gcc ttg cca gag tct cta ccc att   495
Lys Asp Asp Ser Lys His Ala Thr Ala Leu Pro Glu Ser Leu Pro Ile
                120                 125                 130

cat cca ttt ctc acg aca tat cgc gcc gcc atg atg gtt atc acg tgc   543
His Pro Phe Leu Thr Thr Tyr Arg Ala Ala Met Met Val Ile Thr Cys
            135                 140                 145

atc gct atc ttg gct gtg gat ttt cgc att ttt cct cgc cga ttc gcc   591
Ile Ala Ile Leu Ala Val Asp Phe Arg Ile Phe Pro Arg Arg Phe Ala
        150                 155                 160

aag gta gaa aac tgg ggt aca tca ctc atg gat ctg ggc gtt gga tcg   639
Lys Val Glu Asn Trp Gly Thr Ser Leu Met Asp Leu Gly Val Gly Ser
    165                 170                 175

ttt gtc ttt tcg ggc gga gta gta tcc gct cgc tca cta ctc aag agc   687
Phe Val Phe Ser Gly Gly Val Val Ser Ala Arg Ser Leu Leu Lys Ser
180                 185                 190                 195

agg acc aat ggc tct aaa agg ttg cct ctt gcc aag agg ttg att gcg   735
Arg Thr Asn Gly Ser Lys Arg Leu Pro Leu Ala Lys Arg Leu Ile Ala
                200                 205                 210

tcg acg cga cac tct att cct ctg ctc gtc ctc ggc ctg att cgg cta   783
Ser Thr Arg His Ser Ile Pro Leu Leu Val Leu Gly Leu Ile Arg Leu
            215                 220                 225

tac agc gtc aaa ggc ttg gac tat gcg gag cac gtc acc gag tac ggc   831
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Tyr Ser Val Lys Gly Leu Asp Tyr Ala Glu His Val Thr Glu Tyr Gly
        230                 235                 240

gta cat tgg aac ttc ttc ttt aca ttg ggt ctt ttg cct ccg ttc gtg   879
Val His Trp Asn Phe Phe Phe Thr Leu Gly Leu Leu Pro Pro Phe Val
    245                 250                 255

gag gtc ttc gac gcc ttg gct acg atc att ccg tca tac gag gtt ctc   927
Glu Val Phe Asp Ala Leu Ala Thr Ile Ile Pro Ser Tyr Glu Val Leu
260                 265                 270                 275

tcc gtg ggg atc gcc gtc ttg tat caa gtt gcc cta gag tca aca gac   975
Ser Val Gly Ile Ala Val Leu Tyr Gln Val Ala Leu Glu Ser Thr Asp
                280                 285                 290

ttg aaa agc tac atc ctc gtc tcc cct cgt ggg cca agc tta ctg tcc   1023
Leu Lys Ser Tyr Ile Leu Val Ser Pro Arg Gly Pro Ser Leu Leu Ser
            295                 300                 305

aag aat cgt gaa ggc gtc ttc tcc ttc tca ggt tat ctc gcg att ttt   1071
Lys Asn Arg Glu Gly Val Phe Ser Phe Ser Gly Tyr Leu Ala Ile Phe
        310                 315                 320

ctt gct ggt cgt gcg atc ggc att cgg ata atc cct cgc gga act tct   1119
Leu Ala Gly Arg Ala Ile Gly Ile Arg Ile Ile Pro Arg Gly Thr Ser
    325                 330                 335

ttc tca aga agc cca gaa cag gcc agg aga cgg gtc ctg atc agc ctt   1167
Phe Ser Arg Ser Pro Glu Gln Ala Arg Arg Arg Val Leu Ile Ser Leu
340                 345                 350                 355

ggc gtg caa gcg tta gtg tgg acc act ctt ttt gtg ttg aac tcc act   1215
Gly Val Gln Ala Leu Val Trp Thr Thr Leu Phe Val Leu Asn Ser Thr
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tat gcg atg gga tac gga gct aat atc cct gtc tcc cgc cgc ctc gct   1263
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Tyr Ala Met Gly Tyr Gly Ala Asn Ile Pro Val Ser Arg Arg Leu Ala
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aac atg ccc tat gtc ctt tgg gtt tcg gcg ttc aac acc gcg caa ctg  1311
Asn Met Pro Tyr Val Leu Trp Val Ser Ala Phe Asn Thr Ala Gln Leu
        390                 395                 400

ttt gtg ttc tgc ctg atc gaa aca ctc tgc ttt cct gca gtt cat cgg  1359
Phe Val Phe Cys Leu Ile Glu Thr Leu Cys Phe Pro Ala Val His Arg
    405                 410                 415

aca acg act caa gag agc gaa tct gag cga gtc gat ttt gct acg agc  1407
Thr Thr Thr Gln Glu Ser Glu Ser Glu Arg Val Asp Phe Ala Thr Ser
420                 425                 430                 435

cga atc atg tcg gcc ttc aat aag aac agt ctc gcg atc ttt ctt ttg  1455
Arg Ile Met Ser Ala Phe Asn Lys Asn Ser Leu Ala Ile Phe Leu Leu
                440                 445                 450

gcc aat ctt ctg act gga gct gtg aat ctg agc atc tcc aca att gat  1503
Ala Asn Leu Leu Thr Gly Ala Val Asn Leu Ser Ile Ser Thr Ile Asp
            455                 460                 465

gct aat aca gcg cag gcc atc gct gtt ctc att gga tat tca tcc att  1551
Ala Asn Thr Ala Gln Ala Ile Ala Val Leu Ile Gly Tyr Ser Ser Ile
        470                 475                 480

atc aca ggg gtt gct cta gca ttg cat cat gcc aat atc aaa gta ctt  1599
Ile Thr Gly Val Ala Leu Ala Leu His His Ala Asn Ile Lys Val Leu
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Lys Ser Ala Lys Glu Ala Phe Val Ser Asp Asn Pro Gly Ala Ser Ile
  5                  10                  15                  20

tgg agt atc aac gct gtc agc ctg gtc gca ctg gtatgtagct cgttctccga  156
Trp Ser Ile Asn Ala Val Ser Leu Val Ala Leu
                 25                  30

ggggttctgt catttggaga cgcttattaa ttgggatcgc ag gcg aca tat gct     210
                                                Ala Thr Tyr Ala
                                                             35

ctc tgg atc gcc tta tcg ccg tac atc cgt cat gga ctc ctg aac aac    258
Leu Trp Ile Ala Leu Ser Pro Tyr Ile Arg His Gly Leu Leu Asn Asn
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    tac ctg atc tgt gtt ctt ccc cta tta ttc ggg gtg acc atc ttc tca    306
    Tyr Leu Ile Cys Val Leu Pro Leu Leu Phe Gly Val Thr Ile Phe Ser
                55                  60                  65
    act tcg cct ctc gta ttt acc tct ttt ttg tcc att att tcc ctc gct    354
    Thr Ser Pro Leu Val Phe Thr Ser Phe Leu Ser Ile Ile Ser Leu Ala
            70                  75                  80
    ttc atc acg aaa tcc caa aaa tgc ttc aaa tct gtc agt tcg ccc gaa    402
    Phe Ile Thr Lys Ser Gln Lys Cys Phe Lys Ser Val Ser Ser Pro Glu
        85                  90                  95
    aag cca aaa ggc caa tgg cta gac gaa tca gac tcc gat gag gaa cca    450
    Lys Pro Lys Gly Gln Trp Leu Asp Glu Ser Asp Ser Asp Glu Glu Pro
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    gcg gaa cct gct tct gca gct gga tct gca gca gtc tca cca gta aag    498
    Ala Glu Pro Ala Ser Ala Ala Gly Ser Ala Ala Val Ser Pro Val Lys
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    ctt cta cct tcc caa gtg gcg ttc gct tcg gga tcc cta tta tct ccc    546
    Leu Leu Pro Ser Gln Val Ala Phe Ala Ser Gly Ser Leu Leu Ser Pro
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    gat ccg aca aca tcc ccc atg tcg cca agt agt tct tca gct tca gga    594
    Asp Pro Thr Thr Ser Pro Met Ser Pro Ser Ser Ser Ser Ala Ser Gly
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    cat gaa gac cct ttg ggg att atg ggc gtt aac aga cgg agg tcg cta    642
    His Glu Asp Pro Leu Gly Ile Met Gly Val Asn Arg Arg Arg Ser Leu
        165                 170                 175
    tta gaa gga gtt tcg ctt gat gtt ccg tca cat atc gac tcc aag gtc    690
    Leu Glu Gly Val Ser Leu Asp Val Pro Ser His Ile Asp Ser Lys Val
    180                 185                 190                 195
    aga ata tct cct gtt ccc tac ttg agg ctc aaa aag tct agg gca acg    738
    Arg Ile Ser Pro Val Pro Tyr Leu Arg Leu Lys Lys Ser Arg Ala Thr
                    200                 205                 210
    aag gcg caa tgg gtg aaa gaa aag gga aga tta cca ttt ttg aca gtg    786
    Lys Ala Gln Trp Val Lys Glu Lys Gly Arg Leu Pro Phe Leu Thr Val
                215                 220                 225
    tac cga gcg cac atg atg ctc atg act gtt atc tgc atc ttg gcg gta    834
    Tyr Arg Ala His Met Met Leu Met Thr Val Ile Cys Ile Leu Ala Val
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          230                 235                 240
gat ttt gaa gtg ttt cct aga tgg cag ggc aag tgc gaa gat ttt ggt    882
Asp Phe Glu Val Phe Pro Arg Trp Gln Gly Lys Cys Glu Asp Phe Gly
    245                 250                 255
act agt ctg gtaagctttc cttcagccat ggtccagtgc tcaccgctct            931
Thr Ser Leu
260
acttgccgta g atg gac gtg ggt gtc ggg tca ttc gtc ttt tcc ctc ggt    981
             Met Asp Val Gly Val Gly Ser Phe Val Phe Ser Leu Gly
                    265                 270                 275
ctc gtc tcc aca aaa tct ctt tct cct cca cct cca act cct acg ccc   1029
Leu Val Ser Thr Lys Ser Leu Ser Pro Pro Pro Pro Thr Pro Thr Pro
                280                 285                 290
tcc tcg ccc gct ctc aac tct cac atc att ccc ctc acc ccg tcc ccg   1077
Ser Ser Pro Ala Leu Asn Ser His Ile Ile Pro Leu Thr Pro Ser Pro
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ttc act tcc atc ctc atc tcg ctc cga aaa tcc atc ccc atc ctc gtc   1125
Phe Thr Ser Ile Leu Ile Ser Leu Arg Lys Ser Ile Pro Ile Leu Val
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ctc ggc ttt ata cgg ttg att atg gtc aag gga tct gat tat cct gag   1173
Leu Gly Phe Ile Arg Leu Ile Met Val Lys Gly Ser Asp Tyr Pro Glu
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cat gtg acg gag tac ggc gtg cac tgg aat ttc ttc ttc acc ctc gca   1221
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340                 345                 350                 355
ttg gtt cct gtg ctc gcc gtg ggc att cga cca ttg acg cag tgg ctt   1269
Leu Val Pro Val Leu Ala Val Gly Ile Arg Pro Leu Thr Gln Trp Leu
                360                 365                 370
cgc tgg agt gtg ctt ggg gta atc atc tct ttg ctg cat cag ctg tgg   1317
Arg Trp Ser Val Leu Gly Val Ile Ile Ser Leu Leu His Gln Leu Trp
            375                 380                 385
tta aca tat tat ctc caa tcc atc gtc ttc tca ttc ggc cgg tca ggt   1365
Leu Thr Tyr Tyr Leu Gln Ser Ile Val Phe Ser Phe Gly Arg Ser Gly
        390                 395                 400
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Ile Phe Leu Ala Asn Lys Glu Gly Phe Ser Ser Leu Pro Gly Tyr Leu
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     405                 410                 415
tcc ata ttt ttg atc ggc ttg tct att gga gat cat gtt tta agg ctc   1461
Ser Ile Phe Leu Ile Gly Leu Ser Ile Gly Asp His Val Leu Arg Leu
420                 425                 430                 435
agt tta cca cca aga aga gag agg gtc gtg tca gaa aca aat gaa gag   1509
Ser Leu Pro Pro Arg Arg Glu Arg Val Val Ser Glu Thr Asn Glu Glu
                440                 445                 450
cat gag cag agt cat ttt gag aga aaa aaa ttg gat ttg att atg gag   1557
His Glu Gln Ser His Phe Glu Arg Lys Lys Leu Asp Leu Ile Met Glu
            455                 460                 465
ttg att gga tat agc tta ggc tgg tgg gca ctc tta gga ggc tgg att   1605
Leu Ile Gly Tyr Ser Leu Gly Trp Trp Ala Leu Leu Gly Gly Trp Ile
        470                 475                 480
tgg gcc ggc ggg gag gta tcc agg cgt tta gtaagtggac atctttggta     1655
Trp Ala Gly Gly Glu Val Ser Arg Arg Leu
    485                 490
atattgtacc tatactaatc cctgcataaa g gcc aac gct cct tat gta ttt    1707
                                  Ala Asn Ala Pro Tyr Val Phe
                                      495                 500
tgg gta gcg gca tac aat acc acc ttt ctc ctc ggc tac ctc ctc ctt   1755
Trp Val Ala Ala Tyr Asn Thr Thr Phe Leu Leu Gly Tyr Leu Leu Leu
                505                 510                 515
acc cac att att cca tct ccc acc tct tcc caa aca tca cca tcg atc   1803
Thr His Ile Ile Pro Ser Pro Thr Ser Ser Gln Thr Ser Pro Ser Ile
            520                 525                 530
tta gtg cct ccc ttg ctc gac gct atg aat aaa aac ggt ctc gcg ata   1851
Leu Val Pro Pro Leu Leu Asp Ala Met Asn Lys Asn Gly Leu Ala Ile
        535                 540                 545
ttt ttg gcg gcc aac ttg ctt aca gga ctg gtg aat gtg agc atg aag   1899
Phe Leu Ala Ala Asn Leu Leu Thr Gly Leu Val Asn Val Ser Met Lys
    550                 555                 560
aca atg tat gcg ccg gcg tgg ttg tca atg ggg gtg tta atg ttg tat   1947
Thr Met Tyr Ala Pro Ala Trp Leu Ser Met Gly Val Leu Met Leu Tyr
565                 570                 575                 580
acc ttg aca atc agt tgt gta ggg tgg ata ctg aaa gga cgg agg atc   1995
Thr Leu Thr Ile Ser Cys Val Gly Trp Ile Leu Lys Gly Arg Arg Ile
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585 590 595

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Gly Ala Ser Ile Trp Ser Ile Asn Ala Val Ser Leu Val Ala Leu Ala
20 25 30
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Thr Tyr Ala Leu Trp Ile Ala Leu Ser Pro Tyr Ile Arg His Gly Leu
35 40 45
ctg aac aac tac ctg atc tgt gtt ctt ccc cta tta ttc ggg gtg acc 192
Leu Asn Asn Tyr Leu Ile Cys Val Leu Pro Leu Leu Phe Gly Val Thr
50 55 60
atc ttc tca act tcg cct ctc gta ttt acc tct ttt ttg tcc att att 240
Ile Phe Ser Thr Ser Pro Leu Val Phe Thr Ser Phe Leu Ser Ile Ile
65 70 75 80
tcc ctc gct ttc atc acg aaa tcc caa aaa tgc ttc aaa tct gtc agt 288
Ser Leu Ala Phe Ile Thr Lys Ser Gln Lys Cys Phe Lys Ser Val Ser
85 90 95
tcg ccc gaa aag cca aaa ggc caa tgg cta gac gaa tca gac tcc gat 336
Ser Pro Glu Lys Pro Lys Gly Gln Trp Leu Asp Glu Ser Asp Ser Asp
100 105 110

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gag gaa cca gcg gaa cct gct tct gca gct gga tct gca gca gtc tca   384
Glu Glu Pro Ala Glu Pro Ala Ser Ala Ala Gly Ser Ala Ala Val Ser
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cca gta aag ctt cta cct tcc caa gtg gcg ttc gct tcg gga tcc cta   432
Pro Val Lys Leu Leu Pro Ser Gln Val Ala Phe Ala Ser Gly Ser Leu
    130                 135                 140
tta tct ccc gat ccg aca aca tcc ccc atg tcg cca agt agt tct tca   480
Leu Ser Pro Asp Pro Thr Thr Ser Pro Met Ser Pro Ser Ser Ser Ser
145                 150                 155                 160
gct tca gga cat gaa gac cct ttg ggg att atg ggc gtt aac aga cgg   528
Ala Ser Gly His Glu Asp Pro Leu Gly Ile Met Gly Val Asn Arg Arg
                165                 170                 175
agg tcg cta tta gaa gga gtt tcg ctt gat gtt ccg tca cat atc gac   576
Arg Ser Leu Leu Glu Gly Val Ser Leu Asp Val Pro Ser His Ile Asp
            180                 185                 190
tcc aag gtc aga ata tct cct gtt ccc tac ttg agg ctc aaa aag tct   624
Ser Lys Val Arg Ile Ser Pro Val Pro Tyr Leu Arg Leu Lys Lys Ser
        195                 200                 205
agg gca acg aag gcg caa tgg gtg aaa gaa aag gga aga tta cca ttt   672
Arg Ala Thr Lys Ala Gln Trp Val Lys Glu Lys Gly Arg Leu Pro Phe
    210                 215                 220
ttg aca gtg tac cga gcg cac atg atg ctc atg act gtt atc tgc atc   720
Leu Thr Val Tyr Arg Ala His Met Met Leu Met Thr Val Ile Cys Ile
225                 230                 235                 240
ttg gcg gta gat ttt gaa gtg ttt cct aga tgg cag ggc aag tgc gaa   768
Leu Ala Val Asp Phe Glu Val Phe Pro Arg Trp Gln Gly Lys Cys Glu
                245                 250                 255
gat ttt ggt act agt ctg atg gac gtg ggt gtc ggg tca ttc gtc ttt   816
Asp Phe Gly Thr Ser Leu Met Asp Val Gly Val Gly Ser Phe Val Phe
            260                 265                 270
tcc ctc ggt ctc gtc tcc aca aaa tct ctt tct cct cca cct cca act   864
Ser Leu Gly Leu Val Ser Thr Lys Ser Leu Ser Pro Pro Pro Pro Thr
        275                 280                 285
cct acg ccc tcc tcg ccc gct ctc aac tct cac atc att ccc ctc acc   912
Pro Thr Pro Ser Ser Pro Ala Leu Asn Ser His Ile Ile Pro Leu Thr
    290                 295                 300
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Ile Leu Val Leu Gly Phe Ile Arg Leu Ile Met Val Lys Gly Ser Asp
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acc ctc gca ttg gtt cct gtg ctc gcc gtg ggc att cga cca ttg acg   1104
Thr Leu Ala Leu Val Pro Val Leu Ala Val Gly Ile Arg Pro Leu Thr
        355                 360                 365
cag tgg ctt cgc tgg agt gtg ctt ggg gta atc atc tct ttg ctg cat   1152
Gln Trp Leu Arg Trp Ser Val Leu Gly Val Ile Ile Ser Leu Leu His
    370                 375                 380
cag ctg tgg tta aca tat tat ctc caa tcc atc gtc ttc tca ttc ggc   1200
Gln Leu Trp Leu Thr Tyr Tyr Leu Gln Ser Ile Val Phe Ser Phe Gly
385                 390                 395                 400
cgg tca ggt atc ttt cta gca aac aag gaa ggc ttc tcc tct ctt cct   1248
Arg Ser Gly Ile Phe Leu Ala Asn Lys Glu Gly Phe Ser Ser Leu Pro
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Gly Tyr Leu Ser Ile Phe Leu Ile Gly Leu Ser Ile Gly Asp His Val
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Gly Trp Ile Trp Ala Gly Gly Glu Val Ser Arg Arg Leu Ala Asn Ala
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tac ctc ctc ctt acc cac att att cca tct ccc acc tct tcc caa aca   1584
Tyr Leu Leu Leu Thr His Ile Ile Pro Ser Pro Thr Ser Ser Gln Thr
        515                 520                 525
tca cca tcg atc tta gtg cct ccc ttg ctc gac gct atg aat aaa aac   1632
Ser Pro Ser Ile Leu Val Pro Pro Leu Leu Asp Ala Met Asn Lys Asn
    530                 535                 540
ggt ctc gcg ata ttt ttg gcg gcc aac ttg ctt aca gga ctg gtg aat   1680
Gly Leu Ala Ile Phe Leu Ala Ala Asn Leu Leu Thr Gly Leu Val Asn
545                 550                 555                 560
gtg agc atg aag aca atg tat gcg ccg gcg tgg ttg tca atg ggg gtg   1728
Val Ser Met Lys Thr Met Tyr Ala Pro Ala Trp Leu Ser Met Gly Val
                565                 570                 575
tta atg ttg tat acc ttg aca atc agt tgt gta ggg tgg ata ctg aaa   1776
Leu Met Leu Tyr Thr Leu Thr Ile Ser Cys Val Gly Trp Ile Leu Lys
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Thr Tyr Ala Leu Trp Ile Ala Leu Ser Pro Tyr Ile Arg His Gly Leu
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Leu Asn Asn Tyr Leu Ile Cys Val Leu Pro Leu Leu Phe Gly Val Thr
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Ile Phe Ser Thr Ser Pro Leu Val Phe Thr Ser Phe Leu Ser Ile Ile
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Ser Leu Ala Phe Ile Thr Lys Ser Gln Lys Cys Phe Lys Ser Val Ser
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Ser Pro Glu Lys Pro Lys Gly Gln Trp Leu Asp Glu Ser Asp Ser Asp
            100                 105                 110
Glu Glu Pro Ala Glu Pro Ala Ser Ala Ala Gly Ser Ala Ala Val Ser
        115                 120                 125
Pro Val Lys Leu Leu Pro Ser Gln Val Ala Phe Ala Ser Gly Ser Leu
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Leu Ser Pro Asp Pro Thr Thr Ser Pro Met Ser Pro Ser Ser Ser Ser
145                 150                 155                 160
Ala Ser Gly His Glu Asp Pro Leu Gly Ile Met Gly Val Asn Arg Arg
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Arg Ser Leu Leu Glu Gly Val Ser Leu Asp Val Pro Ser His Ile Asp
            180                 185                 190
Ser Lys Val Arg Ile Ser Pro Val Pro Tyr Leu Arg Leu Lys Lys Ser
        195                 200                 205
Arg Ala Thr Lys Ala Gln Trp Val Lys Glu Lys Gly Arg Leu Pro Phe
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Leu Thr Val Tyr Arg Ala His Met Met Leu Met Thr Val Ile Cys Ile
225                 230                 235                 240
Leu Ala Val Asp Phe Glu Val Phe Pro Arg Trp Gln Gly Lys Cys Glu
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Asp Phe Gly Thr Ser Leu Met Asp Val Gly Val Gly Ser Phe Val Phe
            260                 265                 270
Ser Leu Gly Leu Val Ser Thr Lys Ser Leu Ser Pro Pro Pro Pro Thr
        275                 280                 285
Pro Thr Pro Ser Ser Pro Ala Leu Asn Ser His Ile Ile Pro Leu Thr
    290                 295                 300
Pro Ser Pro Phe Thr Ser Ile Leu Ile Ser Leu Arg Lys Ser Ile Pro
305                 310                 315                 320
Ile Leu Val Leu Gly Phe Ile Arg Leu Ile Met Val Lys Gly Ser Asp
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Tyr Pro Glu His Val Thr Glu Tyr Gly Val His Trp Asn Phe Phe Phe
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340 345 350

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Gln Leu Trp Leu Thr Tyr Tyr Leu Gln Ser Ile Val Phe Ser Phe Gly
385 390 395 400

Arg Ser Gly Ile Phe Leu Ala Asn Lys Glu Gly Phe Ser Ser Leu Pro
405 410 415

Gly Tyr Leu Ser Ile Phe Leu Ile Gly Leu Ser Ile Gly Asp His Val
420 425 430

Leu Arg Leu Ser Leu Pro Pro Arg Arg Glu Arg Val Val Ser Glu Thr
435 440 445

Asn Glu Glu His Glu Gln Ser His Phe Glu Arg Lys Lys Leu Asp Leu
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Ile Met Glu Leu Ile Gly Tyr Ser Leu Gly Trp Trp Ala Leu Leu Gly
465 470 475 480

Gly Trp Ile Trp Ala Gly Gly Glu Val Ser Arg Arg Leu Ala Asn Ala
485 490 495

Pro Tyr Val Phe Trp Val Ala Ala Tyr Asn Thr Thr Phe Leu Leu Gly
500 505 510

Tyr Leu Leu Leu Thr His Ile Ile Pro Ser Pro Thr Ser Ser Gln Thr
515 520 525

Ser Pro Ser Ile Leu Val Pro Pro Leu Leu Asp Ala Met Asn Lys Asn
530 535 540

Gly Leu Ala Ile Phe Leu Ala Ala Asn Leu Leu Thr Gly Leu Val Asn
545 550 555 560

Val Ser Met Lys Thr Met Tyr Ala Pro Ala Trp Leu Ser Met Gly Val
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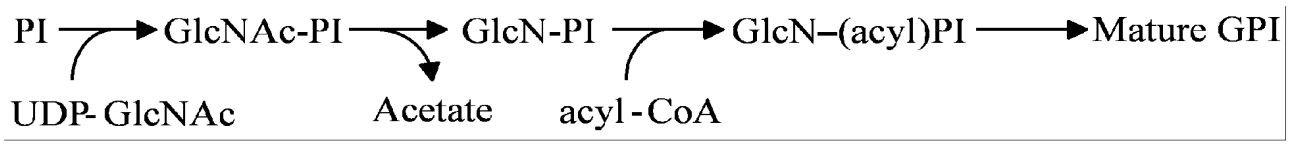
[Brief Description of the Drawings]

[Fig. 1] The GPI biosynthesis pathway is shown.

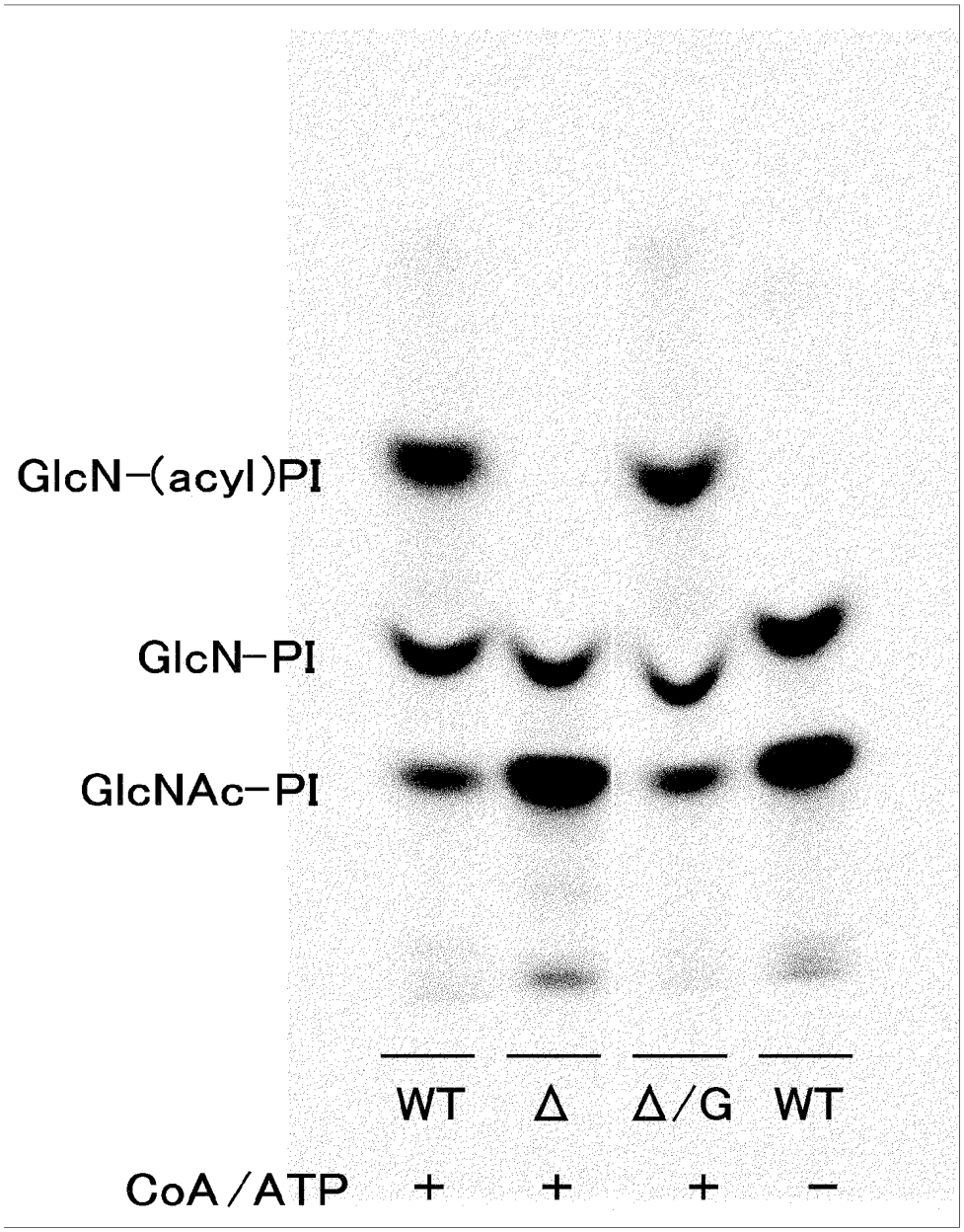
[Fig. 2] A photograph showing the inhibition of binding of labeled CompoundB2 to the membrane fraction by the subject compound is depicted.

[Document Name] Drawings

[Fig. 1]



[Fig. 2]



[Document Name] Abstract

[Abstract]

[Problems to be Solved]

An objective is to develop antifungal agents for preventing pathogenic fungi from exerting pathogenicity, by inhibiting the synthesis of fungal cell walls, as well as by inhibiting fungal cell adhesion to host cells, by inhibition of the transport of GPI-anchored proteins to fungal cell walls.

[Means to Solve the Problems]

The present invention enables screening for compounds that inhibit the transport of GPI-anchored proteins to fungal cell walls, using a simple binding assay using membrane fraction expressing GWT1 protein. New antifungal agents can be created that inhibit the synthesis of fungal cell walls and also inhibit adhesion to host cells by inhibiting the transport of GPI-anchored proteins to fungal cell walls.

[Selected Drawings] None